Scientific paper

The Role of *Fallopia baldschuanica* Plant Extract in the Regression of Induced Hepatocellular Carcinoma in Rats

Luma Abd Almunim Baker,* Shaymaa Zuhir Jalal Aldin and Hamza N Hameed

Department of Chemistry, College of Education for Pure Sciences, University of Mosul, Al-Majmoa'a Street, Mosul 41002, Nineveh, Iraq

** Corresponding author: E-mail: Lumabaker50@uomosul.edu.iq tel.: +9647725380963*

Received: 08-10-2023

Abstract

Liver cancer continues to pose a formidable global health challenge, with its incidence on the rise across the world. Predictions suggest that by 2040, the toll of individuals affected by liver cancer will surpass one million. Consequently, numerous researchers have been motivated to improve therapies and develop new medications with minimal side effects on human health. Plant-derived natural products have offered a variety of pharmacological chemical structures and biologically active substances, which exhibit cytotoxic effects on tumor cells. The current study investigates the potential anti-cancer properties of *Fallopia baldschuanica* flower extract against thioacetamide (TAA) induced cancer. This study pinpointed specific amino acids in the raw extract, with methionine registering the highest percentage, trailed by cysteine, valine, threonine, tyrosine, isoleucine, and lysine. Amino acids have vital activities in various aspects of human health and the condition of diseases. The aim of this study is to evaluate the potential impact of *Fallopia baldschuanica* flower extract on the regression of the experimentally induced HCC in rats. These assessments were conducted through the measurement of liver function, involving aspartate aminotransferase, alanine transaminase, and alkaline phosphatase. Moreover, antioxidant enzyme and tumor marker assays were utilized and the histopathological examination to support the findings.

Keywords: Thioacetamide (TAA); *Fallopian baldschuanica* flower; Mitomycin C; Antioxidant; Anticancer.

1. Introduction

Cancer continues to pose a substantial global health challenge, affecting millions of individuals, with a mortality rate expected to reach approximately 1.4 million by the year 2040.[1] Worldwide, there were approximately 19.3 million new cases of cancer in 2020, with a projected rise to 28.4 million by 2040.[2,3] Various external factors, including smoking, pollution, UV radiation, and chemicals, significantly contribute to this estimate. Additionally, internal factors encompass immunological disorders, genetic mutations, hormones, and alterations occurring during the body's metabolic processes.[4]

The liver is essential for synthesis of bile acids, metabolization of fat, and detoxification.[5] However, the rising tide of metabolic disorders, including metabolic syndrome, diabetes, obesity, and NAFLD, these expected to make individuals more susceptible to hepatocellular carcinoma (HCC).[6,7] Accumulation of fats in the liver is associated with NASH characterized by inflammatory reaction and cellular death, also referred to as NAFLD.[8] Conventional management techniques involving either surgical resection and chemotherapy or liver transplantation have exhibited satisfactory results. Nevertheless, minimizing their side effects would significantly enhance their effectiveness. The above problems underscore the need for other methods that will give secure and more suitable medicines for liver tumor patients.[9]

Oxidative stress takes center stage as a significant mechanism for cancer development. This phenomenon unfolds within an environment where the heightened production of Reactive Oxygen Species (ROS) surpasses the cellular antioxidant defense, creating a pivotal imbalance that contributes to the intricate tapestry of cancer pro-

gression. This oxidative onslaught triggers the oxidation of crucial biomolecules–DNA, proteins, and lipid peroxidation. These oxidative events, fueled by ROS, form a fundamental nexus in the intricate web of cancer formation. ROS was believed to have played an essential role in cancer progression hence the need for novel therapies, and new therapeutic targets.[10]

Carcinogenesis, the intricate process of cancer development, hinges on a diverse array of natural chemicals sourced from plants. This has spurred extensive research into the potential anti-cancer properties of these natural products, positioning plant extracts as indispensable resources for potential cancer chemotherapeutics. In various drug development programs, extracts from plants emerge as critical suppliers, contributing to the expansive arsenal of potential anti-cancer agents.[11] Several plants carry bioactive compounds like alkaloids, flavonoids, terpenoids, and phenolics that possess cytotoxic features towards cancer cells.[12] However, structurally related analogs of those natural substances were developed with different pharmaceutical agents expanding, strengthening the anticancer arsenal.[13] Remarkably, over 70 percent of current anticancer drugs trace their origins to natural resources or their derivatives.[14] This reliance on nature's diversity underscores its significance as a template for discovering novel molecular scaffolds, paving the way for innovative approaches in the ongoing battle against cancer.[15]

Asian *Fallopia baldschuanica* is an herbaceous perennial plant known for its highly competitive and invasive nature within the Polygonaceae family.[16] Studies have revealed its phytochemical composition, highlighting the substantial presence of various compounds, including: anthraquinones (emodin, citreorosein, fallacinol, physcion, and others), flavonoids (rutin, apigenin, quercetin, quercitrin, isoquercitrin, hyperoside, Stilbenes resveratrol and polydatin, coumarins, lignins with essential oil and a host of compounds.[17,18] The key bioactive compounds can be also found in Fallopia plant parts like the stem, leaf, flower, and roots.[19] Extracts obtained from different parts of Fallopia species such as roots, rhizome, stems, leaves, and flowers possess promising medicinal properties including antibacterial[20], antioxidant[21], anticancer, antiproliferative and apoptotic properties.[22] These findings emphasize the capacity of Fallopia species to serve as a rich source of diverse natural compounds with potential therapeutic applications. For instance, a previous study illustrated that *Fallopia japonica* extract exhibited potent inhibition of ABC transporters, significant inhibition of metabolic enzymes, and cell growth.[23] As research continues, natural products may prove to be valuable sources of medication regarding those with cancer, with the ability to offer safer and more effective therapies.[24]

The aim of the present study is to evaluate the potential anticancer effects of *Fallopia baldschuanica* flower extract on thioacetamide D (TAA)-induced cancer. For this purpose, the anticancer potential of plant extract was examined through biochemical studies of liver function tests and histological examination.

2. Experiment

2. 1. Preparation of the Plant Extracts

Asian *Fallopia baldschuanica* was used in this study, Family: Polygonaceae, Genus: *Fallopia baldschuanica* and Species: *F. baldschuanica*.[25]

Asian *Fallopia baldschuanica* flower (100 g) was taken and homogenized after mixing with distilled water (1:5) weight: volume and then the crude extract was prepared.[26]

2. 2. Experimental Design

In the present study, male rats (Wister) weighing between 200 and 250 gm of body weight were involved in this study. For the experiments on rats, the study received approval from the University of Mosul College of Veterinary Medicine/ Institutional Animal Care and Use Committee, under reference number UM.VET.2023.029. This rigorous ethical oversight ensures the humane and responsible treatment of the animal subjects involved in the research, upholding the principles of ethical conduct in scientific investigations. To ensure methodological precision and mitigate potential complications, a deliberate decision was made to exclude the influence of the female rat estrus cycle, which could introduce variability in the results. 36 rats were involved in the study, which was divided into 6 groups with 6 animals in each group. All groups were treated as the following:

Group 1: Control group (Normal). These rats were given a regular diet with distilled water for 48 days.

Group 2: a group that received 23 days of treatment with flower plant extract.

Group 3: a group that received a daily LD_{50} dose of 100 mg/kg of TAA for 5 days. The 50% Lethal Dose (LD_{50}) was determined using the methodology outlined in the study conducted by Enegide *et al.*[27] The specific concentration of TAA was diluted (200, 175, 150, 125, 100, 75, and 50 mg/kg), ten male rats for each concentration. The LD_{50} value, a critical measure of lethal dose, is typically derived using a straightforward formula:

LD_{50}=[M0+M1]/2, where M0 represents the highest administered dose with no mortality, while M1 signifies the lowest dose at which mortality is observed.

Group 4: a group that received daily doses of TAA for five days and then treated with flower plant extract for a period of 23 days.

Group 5: a group that received daily doses of TAA for five days and then treated with mitomycin C (MMC) for a period of 23 days.

Group 6: a group that received daily doses of TAA for five days. After that, they were treated with a combination of flower plant extract and MMC for a duration of 23 days.

After the specified period, blood samples were collected from the medial canthus of the eye of male rats to conduct the required tests.[28] Then after a period of 30 days, the rats were dissected to examine the extent of liver damage caused by TAA. Liver samples were extracted, weighed, and then fixed in a 10% formalin solution for further analysis.

2. 3. Histology

Liver tissue has been fixed in 10% formalin and then dehydrated using an ethanol gradient. Washing has been done with xylene and embedded in paraffin wax. The tissue blocks were sectioned at a thickness of 5–6 micrometers, deparaffinized, and stained with hematoxylin and eosin to enable microscopic examination.[29]

2. 4. Biochemical Parameters Assay

Serum samples were analyzed for various biochemical parameters, including alpha-fetoprotein AFP measured using an ELISA kit (Kamiya Biomedical),[30] alkaline phosphatase ALP measured using an ELISA kit (Mybiosource),[31] Aspartate Transaminase (AST) measured using an ELISA kit (Mybiosource),[26] alanine transaminase (ALT) measured using an ELISA kit (Mybiosource),[32] Malondialdehyde (MDA) levels, which were determined using a modified thiobarbituric acid reaction.[34] The level of glutathione (GSH) was measured using Ellman's reagent/ DTNB,[34] while the total protein levels were determined using the Biuret method at 564nm.[35]

2. 5. Amino Acid Analyzer

Amino acids were extracted from the sample using the Young Lin Amino Acid Analysis System, which employed a ZORBAX Eclipse-AAA column for separation. The column had a dimension of 150 × 4.6 mm with a particle size of 3.5 µm. This system was available at the Ministry of Science and Technology/Department of Environment and Water Laboratories.[36]

2. 6. Statistical Analysis

The acquired data underwent meticulous statistical scrutiny employing one-way analysis of variance (ANOVA). To discern specific differences between the groups, the Duncan test was employed, providing a detailed exploration of the dataset. All statistical computations were executed with the aid of IBM SPSS Statistics 22 software. Additionally, Graph Pad Prism v8.0 was used for graphical representation. The significance level for the statistical analysis was set at 5% ($P < 0.05$).

3. Results and Discussion

The liver dysfunction is a major health problem. The present study evaluates the potential role of *Fallopia baldschuanica* flower extracts on thioacetamide D (TAA)-induced cancer in rats. TAA has been widely used by researchers as an experimental model to induce liver damage in animals. The toxicity of TAA causes oxidative stress leading to the production of reactive oxygen species, inflammation responses, and apoptosis in Hepatocytes. This ultimately leads to liver injury and failure. Consequently, there is an elevation in serum aminotransferase levels, including aspartate aminotransferase and alanine aminotransferase (ALT).[37]

3. 1. The effect of Some Enzymes on Liver Cancer

Liver enzymes are essential for detoxification purposes yet an imbalance in their levels leads to liver damage that causes initiation, progression, and spread of liver cancer. The most sensitive biochemical indicators used to diagnose hepatic impairment are serum AST, ALT, and ALP.[38]

The mean of AST, ALT, and ALP levels in serum were significantly ($P < 0.05$) higher in TAA-treated rats than in the control group 92.75, 61.75, 63.3 to 117.5, 155.75, and 153.69 respectively (Fig. 1; Table 1). There were significantly increased concentrations of AST and ALT in TAA-treated male rats but given extract crude of flower plant when compared to the group given crude extract only. Abnormally high levels of these enzymes denote the presence of liver damage, inflammation, or tumor growth (1, 2). These findings are in agreement with earlier works that showed similarly raised levels of liver enzymes.[37,39,40] The graph depicted in Figure shows a statistically viable reduction ($P < 0.05$) of these mentioned factors for the male rats receiving TAA with MMC, flower plant extract, or MMC + flower plant extract compared to the male rats treated with the TAA group (Fig. 1; Table 1). These results are consistent with Marzouk *et al.* (2011) findings, which demonstrated decreased activities of certain enzymes (AST, ALT, and ALP) associated with liver damage in male rats treated with the hydroalcoholic extract of *Cichorium endivia*.[41] This indicates the potential of the plant extract to alleviate liver damage in TAA-treated rats. Furthermore, the administration of MMC resulted in a decrease in ALT and ALP activities, which indicates an improvement in liver function.[42,43] Many studies demonstrated that certain plant extracts can exhibit synergistic effects when used in combination with conventional chemotherapy drugs, Consequently, their anticancer properties would take effect.[44] Various plant extracts with different structures have demonstrated efficacy in reversing various malignancies.

According to a recent study, the combination of *C. maritimum* ethyl acetate extract and a half-dosage of sorafenib IC_{50} reduced the suppression of HCC cell lines (Huh7 and HepG2) growth comparably to a full dose of sorafenib, without causing more cell damage.[45] In contrast, there was a significant ($P < 0.05$) increase in the activity of

AST observed in the TAA male rats group when treated with MMC, as depicted in Figure 1 and Table 1. The reason may be attributed to the rapid destruction of cancer cells during treatment can lead to increased AST levels due to the release of AST from the dying cells.

Notably, the most potent agent in inhibiting the growth of HCC was an ethyl acetate fraction derived from extracts of *Brassica oleracea L.* and *Crithmum maritimum L.* This mechanism resulted in the inhibition of protein synthesis, thereby influencing membrane biosynthesis, and disrupting the lipid equilibrium within HCC cells. All these items played a significant part in enhancing chemotherapy and reducing its side effects.[46–49]

Notably, the protein content demonstrated minimal variation between TAA-treated male rats and the healthy control group. This aligns with a prior study, where no statistical difference in serum total protein levels was observed among patients with oral cancer.[50] Nevertheless, this finding differs from other studies dealing with low levels of total protein in cancer.[51, 52]

There was a statistically significant decrease observed ($P < 0.05$) for the AFP levels among TAA-treated rats with flower plant extract, MMC, or MMC+flower plant extract compared to the TAA group (Fig. 1; Table 2). The decrease can be attributed to some of the components found in the plant flower extract, including antioxidants and essential amino acids that are known to enhance hepatic functionality and may buffer the effects of thioacetamide.[53,54]

Table 1: Effects sum of AST, ALT, and ALP in both normal and experimental conditions.

	Control	Crude extract	TAA	TAA + crude extract	TAA + MMC	TAA + MMC + crude extract
AST	92.75 ± 2.50	54.25 ± 3.77*	117.50 ± 2.08*	94.25 ± 2.5	131.50 ± 7.77*	45.50 ± 7.05*
	c	b	d	c	e	a
ALT	61.75 ± 8.02	59.00 ± 9.20	155.75 ± 4.35*	72.75 ± 4.57*	63.50 ± 4.80	90.50 ± 2.08*
	a	a	d	b	a	c
ALP	63.30 ± 4.26	71.51 ± 8.89*	153.69 ± 3.97*	8.02 ± 0.51*	5.91 ± 0.89*	5.63 ± 1.00*
	b	c	d	a	a	a

AST=aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; MMC= Mitomycin C
Values are given as mean± SD of 5 replicates; *P value <0.05 = significant level.
The variables a, b, c, d, and e are represented in multiple comparison settings among groups that were identified using the Duncan test.

3. 2. The Effect of Some Biochemical Parameters on Liver Cancer

The findings of this study highlight a significant elevation in serum AFP and MDA levels in TAA-treated male rats, registering at 186.19 and 6.13, respectively, compared to the control group, which exhibited levels of 2.11 for AFP and 1.65 for MDA (Fig. 1; Table 2). This notable surge in oxidative stress is attributed to lipid peroxidation within liver tissue, a consequence of TAA-induced hepatotoxicity.

The concentration of TP in the TAA-treated rats considerably dropped from 17.56 g/dL to 8.66 g/dL in comparison to the control rats treated with floral plant extract, as evidenced by the results in Fig. 1 and Table 2. This decline is a result of the body's inability to properly digest proteins because of TAA exposure, which might result in hypoalbuminemia. The prevailing cirrhotic state associated with malabsorption is known to contribute to

Table 2: Effects sum of biochemical variables in both normal and experimental conditions.

	Control	Crude extract	TAA	TAA + crude extract	TAA + MMC	TAA + MMC + crude extract
AFP ng/ml	2.11 ± 0.35	2.94 ± 0.87	186.19 ± 6.51*	144.85 ± 14.53*	33.46 ± 1.94*	69.76 ± 6.98*
	a	a	e	d	b	c
T.P g/dL	9.67 ± 0.92	17.42 ± 2.24*	12.17 ± 0.24	8.66 ± 1.00	16.52 ± 1.68*	14.04 ± 3.61*
	ab	e	bc	a	de	cd
GSH $*10^{-6}$ µmol/L	3.35 ± 0.49	2.55 ± 0.16*	1.29 ± 0.05*	2.46 ± 0.38*	2.50 ± 0.20*	2.70 ± 0.10*
	c	b	a	b	b	b
MDA $* 10^{-5}$ µM/L	1.71 ± 0.04	2.38 ± 0.14*	6.60 ± 0.51*	4.83 ± 0.11*	4.93 ± 0.24*	5.81 ± 0.10*
	a	b	e	c	c	d

AFP = alpha-fetoprotein; TP= total protein; GSH = glutathione; MDA= malondialdehyde.
Values are given as mean± SD of 5 replicates; *P value < 0.05 = significant level.
The variables a, b, c, d, and e are represented in multiple comparison settings among groups that were identified using the Duncan test.

decreased albumin levels.[55] In alignment with this, the study validates a substantial increase in total protein (TP) concentration in TAA-treated rats receiving injections of MMC, rising significantly from 12.17 g/dL to 16.52 g/dL compared to the TAA-only group. However, the findings revealed a non-significant increase in the concentration

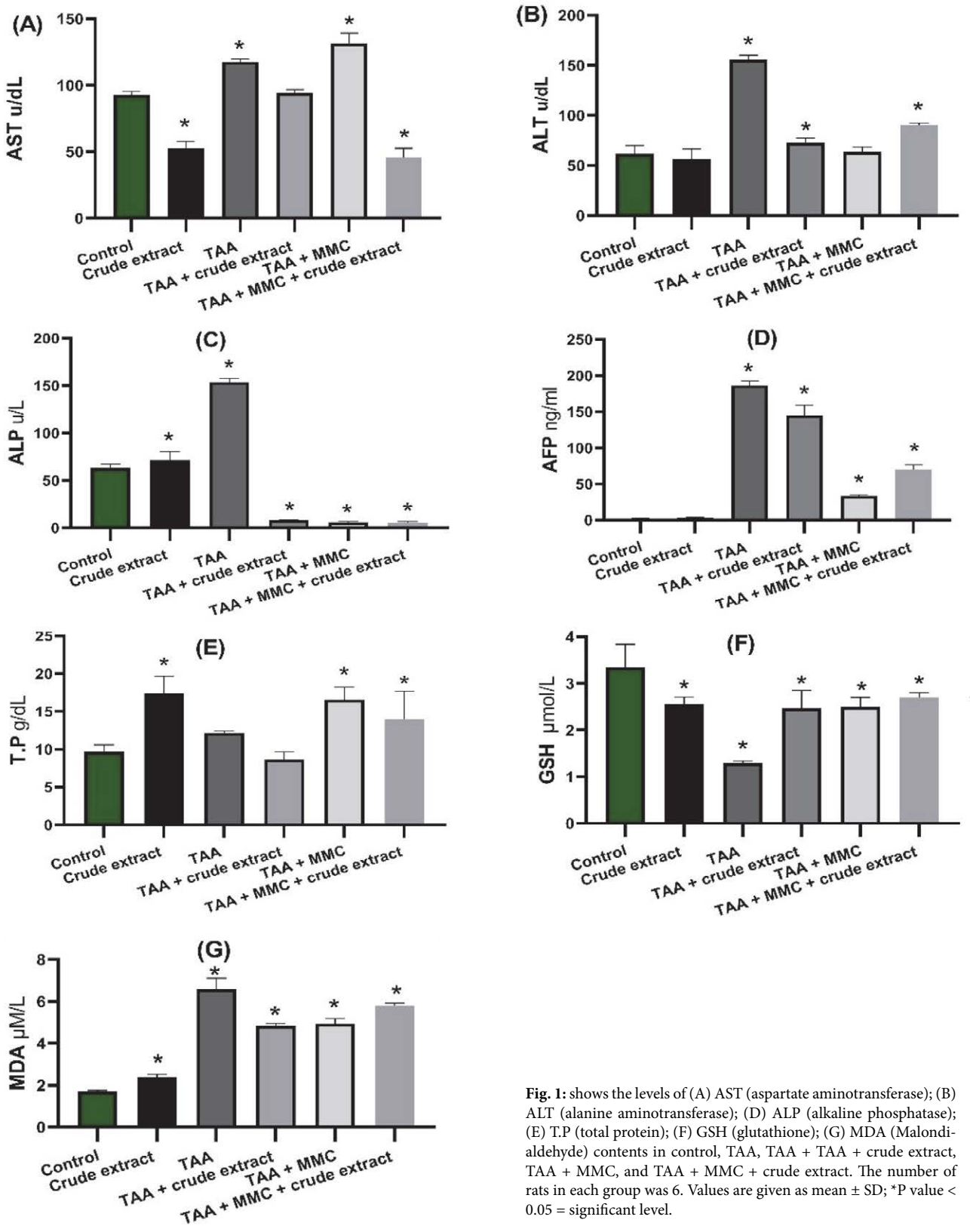

Fig. 1: shows the levels of (A) AST (aspartate aminotransferase); (B) ALT (alanine aminotransferase); (D) ALP (alkaline phosphatase); (E) T.P (total protein); (F) GSH (glutathione); (G) MDA (Malondialdehyde) contents in control, TAA, TAA + TAA + crude extract, TAA + MMC, and TAA + MMC + crude extract. The number of rats in each group was 6. Values are given as mean ± SD; *P value < 0.05 = significant level.

of total protein (TP) following treatment with a combination of MMC and the flower plant extract. The exposure of MMC in cancer cell exposure is known to trigger the DNA damage response (DDR), a cellular mechanism.[56] One protein that can be stimulated by the DDR is p53, a transcription factor controlling the expression of various genes related to DNA repair, cell cycle, and apoptosis. Activation of p53, in turn, can potentially elevate the overall cellular protein levels, presenting a complex interplay of molecular events in response to the treatment.[56]

In contrast, the data showed a significant reduction in total protein (TP) activity among male rats treated with both TAA (thioacetamide) and the flower plant extract, as opposed to the TAA group. Natural products employed in cancer treatment can decrease the proteins associated with tumor growth by lowering their expression levels. This process leads to apoptosis and decreased tumor growth.[57]

According to the data shown in Fig. 1 and Table 2, rats treated with flower plant extract, MMC or a combination of both demonstrated reduced levels of MDA compared to the group that received only TAA. Remarkably, TAA-treated male rats receiving the flower plant extract displayed significantly elevated MDA levels compared to the control group treated solely with the extract. MDA, a widely recognized biomarker of oxidative stress in cancer, and the observed reduction in MDA levels implies a potential protective effect against the oxidative stress induced by TAA.

In Table 2, the data shows serum Glutathione (GSH) levels of the untreated control group exhibited significantly higher GSH levels at 3.34 μmol/L compared to the TAA-treated male rats of 1.29 μmol/L. Interestingly, the GSH levels in the male rats treated with TAA and administrated with flower plant extract, as well as the control rats administrated with flower plant extract, showed no significant difference. Furthermore, the GSH data have revealed a significant elevation in TAA-treated rats administered with various treatments of (MMC, MMC + flower plant extract, or flower plant extract) when contrasted with the TAA-treated male rats. GSH is one of the significant antioxidants that assumes a crucial role in shielding cells from the detrimental impact of free radicals. Nevertheless, flower plant extract and MMC increased the activity of GSH indicating a positive effect against oxidative stress.[57]

3. 3. Estimation of Amino Acids in the *Fallopia baldschuanica* Crude Extracts

The crude extracts of *Fallopia baldschuanica* flower reveal the presence of various amino acids, including glutamic acid, valine, glycine, threonine, serine, isoleucine, and phenylalanine, that may have antioxidant properties, helping to eliminate harmful free radicals within the body (Fig. 2; Table 3). Notably, methionine constitutes a larger percentage of the sample, playing a pivotal role in bolstering antitumor immunity by enhancing the activity of cyclic GMP-Amp synthases and promoting chromatin dissociation.[58,59] Similarly, cysteine, another amino acid identified in the extract, is recognized as a tumor regression amino acid, that enhances chemotherapy.[60]

3. 4. Histological Examination

Hematoxylin and eosin (H&E) staining has been used to examine liver tissue under a microscope. According to the description provided, the control group showed normal architecture of the hepatic lobules. Microscopical examination of liver sections stained with H&E, the control group exhibited a normative architectural profile with

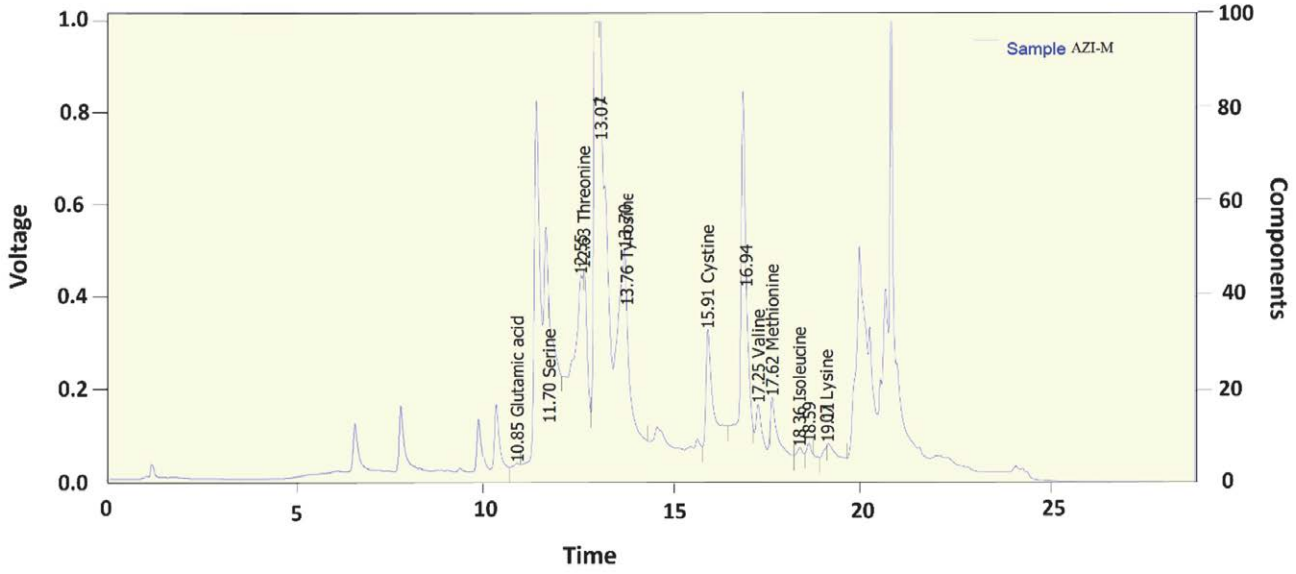

Fig. 2: Amino acids identified in the crude extracts of *Fallopia* flower.

Table 3: Percentage of amino acids present in the crude extracts of *Fallopia* flower.

	Reten. Time [min]	Response	Amount [ppm]	Amount [%]	Peak Type	Compound Name
1	10.848	37.642	1.122	0.5	Ordnr	Glutamic acid
2	11.704	207.714	2.125	1.0	Ordnr	Serine
3	12.548	3251.788	0.000	0.0		
4	12.632	1853.310	20.134	9.6	Ordnr	Threonine
5	13.012	7948.690	0.000	0.0		
6	13.072	12747.388	0.000	0.0		
7	13.700	2944.959	0.000	0.0		
8	13.760	1635.572	19.948	9.5	Ordnr	Tyrosine
9	15.912	3528.679	58.573	27.8	Ordnr	Cysteine
10	16.844	6577.316 j	0.000	0.0		
11	16.936	2009.170	0.000	0.0		
12	17.248	1256.845	26.694	12.7	Ordnr	Valine
13	17.616	1308.515	69.785	33.2	Ordnr	Methionine
14	18.356	132.608	6.149	2.9	Ordnr	Iso leucine
15	18.592	161.316	0.000	0.0		
16	19.068	151.532	5.862	2.8	Ordnr	Lysine
17	19.108	365.632 1	0.000	0.0		
	Total		210.392	100.0		

a notable proliferation of Kupffer Cells, signaling the absence of any discernible pathological changes in the tissue (Fig. 3). The microscopic portrayal of the TAA group (100 mg/kg of TAA over 5 days) showed intricate pathological alterations. Hepatocellular carcinoma, sinusoidal dilatation, the penetration of mononuclear cells, degeneration

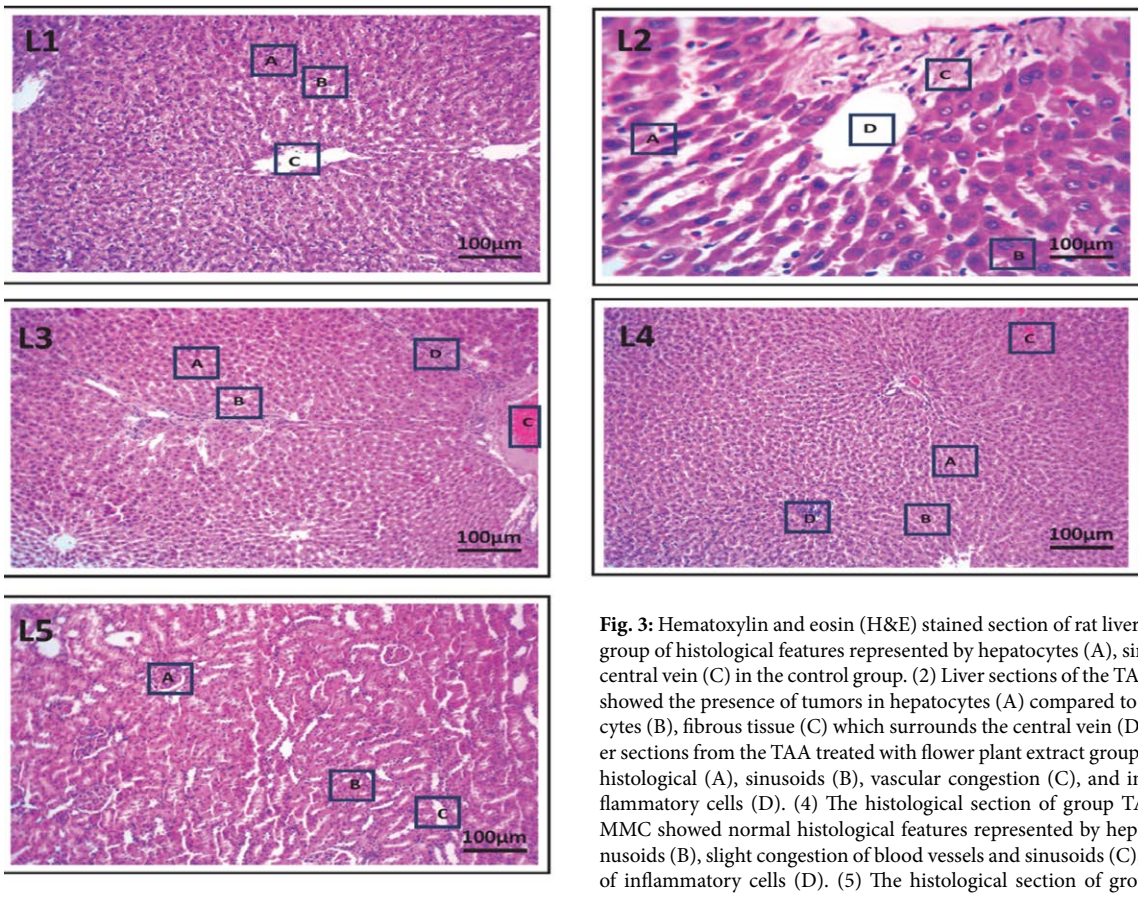

Fig. 3: Hematoxylin and eosin (H&E) stained section of rat liver. (1) The control group of histological features represented by hepatocytes (A), sinusoids (B), and central vein (C) in the control group. (2) Liver sections of the TAA-treated group showed the presence of tumors in hepatocytes (A) compared to normal hepatocytes (B), fibrous tissue (C) which surrounds the central vein (D), 400X. (3) Liver sections from the TAA treated with flower plant extract group showed normal histological (A), sinusoids (B), vascular congestion (C), and infiltration of inflammatory cells (D). (4) The histological section of group TAA treated with MMC showed normal histological features represented by hepatocytes (A), sinusoids (B), slight congestion of blood vessels and sinusoids (C), and infiltration of inflammatory cells (D). (5) The histological section of group TAA-treated male rats with flower plant extract and MMC showed the normal histological features represented by the central vein (A), the sinusoids (B), and a slight (cloudy) vacuolar degeneration of the hepatocytes (C).

of hepatocytes, cellular hypertrophy, and vascular congestion are all included (Fig. 3). Moreover, the TAA-induced liver injury precipitated an increase in ROS production, instigating oxidative stress and damage to proteins, lipids, and DNA within the hepatic cellular milieu. This is consistent with previous studies which indicated that the use of the chemical substance TAA caused liver tumors and infiltration of hepatocytes.[61,62]

The lesions were reduced in a TAA group treated with the flower plant extract and only a few tumor cells were present. These findings indicate that the plant extract may have anti-cancer properties that can effectively reduce the development of tumors in liver tissue (Fig. 3).

The histological sections of TAA group treated with MMC at a concentration of 75 mg/kg (which is used as chemotherapy) showed changes in the liver tissue, including the appearance of some tumor cells, nucleus polymorphism and division, and the emergence of congestion in the central vein of the liver, and infiltration of inflammatory cells (Fig. 3). Many studies suggest that MMC can effectively shrink tumors when used as a form of chemotherapy. However, the use of MMC can also lead to various side effects that affect the liver tissue, such as congestion in the central vein and infiltration of hepatocytes.[63]

Histological sections revealed that treatment with both MMC and plant extract induced changes in the liver tissue. These changes included less appearance of tumor cells, the proliferation of Kupffer cells, and sinusoidal obstruction (Fig. 3). These findings suggest that the combination of MMC and plant extract may have an impact on liver health, affecting the liver tissue structure and function. Many studies have revealed that *Annona muricata* plant pulp has several beneficial effects on liver tissue, including anti-cancer properties attributed to the presence of flavonoids and phenols.[64]

4. Conclusion

The findings of the current study have reinforced the idea that *Fallopia baldschuanica* flower crude extracts or combining them with MMC can effectively improve liver damage caused by TAA-treated rats. The data demonstrated that the plant extract improved the reversal of liver damage caused by TAA, through the restoration of liver enzyme levels that approach normal. Furthermore, this study has provided new insights into the potential benefits of this combination therapy. However, further research is required to fully understand the mechanisms behind how these substances work together.

Acknowledgments

We would like to extend our heartfelt gratitude and appreciation to Mosul University/College of Education for Pure Science/Department of Chemistry for the unwavering support and exceptional facilities provided throughout our study period.

The source of funding was provided through self-financing.

5. References

1. WHO, Number of new cases and deaths from liver cancer predicted to rise by more than 55% by 2040 (who.int), (6 October 2022).
2. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, *Cancer J. Clin*. **2018**, *68,* 3–31. **DOI:**10.3322/caac.21492
3. H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, *Cancer J. Clin*. **2021**, *71,* 209–249. **DOI:**10.3322/caac.21660
4. M. Maule, F. Merletti, *Oncology*. **2012**, *13,* 745–746. **DOI:**10.1016/S1470-2045(12)70268-1
5. E. Trefts, M. Gannon, D. H. Wasserman, *Curr. Biol*. **2017**, *27*, 1147–1151. **DOI:**10.1016/j.cub.2017.09.019
6. A. Forner, M. Reig, J. Bruix, *Lancet*. **2018**, *391*, 1301–1314. **DOI:**10.1016/S0140-6736(18)30010-2
7. P. Agosti, C. Sabba, A. Mazzocca, *Bba-Mol. Basis Dis*. **2018**, *1864*, 607–617. **DOI:**10.1016/j.bbadis.2017.11.026
8. D. Gnocchi, C. Sabbà, M. Massimi, A. Mazzocca, *Int. J. Mol. Sci*. **2023**, *24*, 1–17. **DOI:**10.3390/ijms24043710
9. C. Y. Liu, K. F. Chen, P. J. Chen, *Csh. Perspec.t Med*. **2015**, *5,* 1–16. **DOI:**10.1101/cshperspect.a021535
10. K. M. Zahra, R. Lefter, A. Ali, H. A. Ech-Cha, T. Constantin, C. Alin, T. Daniel, *Oxid. Med. Cell Longev*. **2021**, *5,* 1–25. **DOI:**10.1155/2021/9965916
11. B. Orlikova, M. Diederich, *Curr. Med. Chem*. **2012**, *19,* 2061–2087. **DOI:**10.2174/092986712800228998
12. M. Singh, M. Kaur, O. Silakari, *Eur. J. Med. Chem*. **2014**, *84,* 206–239. **DOI:**10.1016/j.ejmech.2014.07.013
13. M. Gordaliza, *Clin. Transl. Oncol*. 2007, *9,* 767–776. **DOI:**10.1007/s12094-007-0138-9
14. G. A. Karikas, *J. BUON*. **2010**, *15,* 627–638. **DOI:**10.3390/molecules15020627
15. G. Tan, C. Gyllenhaal, *Curr. Drug Targets*. **2006**, *7,* 265–277. **DOI:**10.2174/138945006776054942
16. J. Forman,R. V. Kesseli, *Am. J. Bot*. 2003, *90*, 586–592. **DOI:**10.3732/ajb.90.4.586
17. J. H. Park, H. K. Moon, C. W. Park, *Korean J. Plant Taxon*. **2011**, *41*, 10–15. **DOI:**10.11110/kjpt.2011.41.1.010
18. S. Lachowicz, J. Oszmianski, *Molecules*. **2019**, *24*, 1436. **DOI:**10.3390/molecules24071436
19. V. Metlicar, I. Vovk, A. Albreht, *Plants*. **2019**, *8*, 1–15. **DOI:**10.3390/plants8100384
20. I. Nawrot-Hadzik, J. Hadzik, M. Fleischer, A. Choromanska, B. Sterczała, P. Kubasiewicz-Ross, J. Saczko, M. Gałczynska-Rusin, T. Gedrange, A. Matkowski, *Med. Sci. Monit*. **2019**, *25,* 3279–3287. **DOI:**10.12659/MSM.913855
21. C. Chan, R. Gan, H. Corke, *Int. J. Food Sci. Tech*. **2016**, *51*, 565–573. **DOI:**10.1111/ijfs.13024

22. M. Hong, H. Y. Tan, S. Li, F. Cheung, N. Wang, T. Nagamatsu, Y. Feng, *Int. J. Mol. Sci*. **2016**, *17*, 893. 1–21. **DOI:**10.3390/ijms17060893
23. S. Y. Eid, M. Z. El-Readi, M. L. Ashour, M. Wink, *Evid-Based Compl. Alt*. **2015**, *2015*, pp, 1–8. **DOI:**10.1155/2015/868424
24. S. Mishra, S. Ahmad, N. Kumar, B. Sharma, *Glob. J. Pharma Res*. **2013**, *2,* 1613–1618.
25. M. H. Kim, J. H. Park, H. P. Won, W. Chong, *Can. J. Bot*. **2000**, *78,* 1136–1143. **DOI:**10.1139/b00-079
26. E. Agbai, C. Njoku, C. Nwanegwo, *Afr. J. Pharm. Pharmaco*. **2015**, *9,* 745–755. **DOI:**10.5897/AJPP2015.4389
27. C. Enegide, A. David, S. A. Fidelis, *Toxicol. Int*. **2013**, 20,224–226. **DOI:**10.4103/0971-6580.121674
28. D. Stillinger, K. Helland, C. Van Atta, *J. Fluid. Mech*. **1983**, *131*, pp, 91–122. **DOI:**10.1017/S0022112083001251
29. N. Aljuhani, M. A. Elkablawy, H. M. Elbadawy, A. M. Alahmadi, A. M. Aloufi, S.H. Farsi, B. S. Alhubayshi, S. S. Alhejaili, J. M. Alhejaili, O. B. Abdel-Halim, *J Taibah Univ. Med. Sci*. **2019**, *14,* 553–559. **DOI:**10.1016/j.jtumed.2019.10.002
30. J. Liang, J. Wang, L. Zhang, S. Wang, C. Yao, Z. Zhang, *Analyst*. **2019**, *144,* 265–273. **DOI:**10.1039/C8AN01791C
31. M. Tomás, M. Sentí, F. n. García-Faria, J. Vila, A. Torrents, M. Covas, J. Marrugat, *Arterioscl. Throm. Vas*. **2000**, *20,* 2113–2119. **DOI:**10.1161/01.ATV.20.9.2113
32. I. S. Khan, K. B. Dar, S. A. Ganie, M. N. Ali, *Drug Chem. Toxicol*. **2020**, *45,* 1345–1354. **DOI:**10.1080/01480545.2020.1825472
33. N. A. Botsoglou, D. J. Fletouris, G. E. Papageorgiou, V. N. Vassilopoulos A. J. Mantis, A. G. Trakatellis, *J. Agr. Food Chem*. **1994**, *42,* 1931–1937. **DOI:**10.1021/jf00045a019
34. S. Cao, X. Li, Y. Gao, F. Li, K. Li, X. Cao, Y. Dai, L. Mao, S. Wang, X. Tai, *Dalton T*. **2020**, *49,* 11851–11858. **DOI:**10.1039/D0DT01742F
35. A. G. Gornall, C. J. Bardawill, M.M. David, *J. Biol. Chem*. **1949**, *177,* 751–766. **DOI:**10.1016/S0021-9258(18)57021-6
36. R. Dahl-Lassen, J. van Hecke, H. Jørgensen, C. Bukh, B. Andersen, J. K. Schjoerring, *Plant Methods*. 2018, 14, 1–9. **DOI:**10.1186/s13007-018-0277-8
37. S. Y. Nancy, M. H. Amal, A. G. Mohammad, Elmorsy, A. M. Heba, *Sci. Rep-UK*. **2021**, *11,* 1–17. **DOI:**10.1038/s41598-021-91666-6
38. N. Johnkennedy, E. Adamma, A. Austin, N. N. Chukwunyere, *Am. J. Sci*. **2010**, *3,* 317–321.
39. M. Bhadauria, S. K. Nirala, *Food Chem. Toxicol*. **2009**, *27,* 17–25. **DOI:**10.1016/j.etap.2008.07.003
40. S. H. Shareef, A. S. M. Juma, D. N. F. Agha, A R. Alzahrani, I. A.A. Ibrahim, M. A. Abdulla, *Appl. Sci.* **2023**, *13,* 5243, 2–19. **DOI:**10.3390/app13095243
41. M. Marzouk, A. A. Sayed, M. A. Soliman, *J Med*. **2011**, *2,* 1273–1279.
42. C. A. Ukwubile, *Int. J. Pharm. Front*. **2012**, *2,* 9–17. **DOI:**10.1016/S1995-7645(14)60258-3
43. S. H. Orabi, S. M. Shawky, *Int. J. Sci. Basic Appl. Res*. **2014**, *17,* 137–147.
44. S. Pezzani, I. Vitalini, S. R. Zuñiga, M. Martorell, *Medicina*. **2019**, *55,* p. 110. **DOI:**10.3390/medicina55040110
45. D. Gnocchi, F. Castellaneta, G. Cesari, G. Fiore, C. Sabbà, A. Mazzocca, *J. Pharm. Pharmacol*. **2012**, 73, 1369–1376. **DOI:**10.1093/jpp/rgab070
46. D. Gnocchi, G. Cesari, G. J. Calabrese, R. Capone, C. Sabbà, A. Mazzocca, *Plant Food Hum. Nutr*. 2020, 75, pp, 33–40. **DOI:**10.1007/s11130-019-00781-3
47. D. Gnocchi, L. Del Coco, C. R. Girelli, F. Castellaneta, G. Cesari, C. Sabbà, F. P. Fanizzi, A. Mazzocca, *Sci. Rep. UK*. 2021. 11, p.1259. **DOI:**10.1038/s41598-020-78867-1
48. D. Gnocchi, C. Sabbà, A. Mazzocca, *Plant Food Hum. Nutr*. 2022, 77, 481–483. **DOI:**10.1007/s11130-022-00986-z
49. D. Gnocchi, C. Sabbà, A. Mazzocca, *Plant Food Hum. Nutr*. 2023, 78, 230–232. **DOI:**10.1007/s11130-022-01037-3
50. N. Abhishek, K. Mubeen, K. R. Vijayalakshmi, B. Suman, H. C. Gayitri, M. Anitha, *Malaysian J. Pathol*. **2012**, *34,* 4 –52.
51. M. Rasouli, A. Okhovatian, A. Enderami, *Clin. Chem. Lab. Med*. **2005**, *43,* 913–918. **DOI:**10.1515/CCLM.2005.156
52. S. AlMuhtaseb, *Oncol. Lett*. **2014**, *8,* 2752–2756. **DOI:**10.3892/ol.2014.2535
53. A. M. Gowifel, M. G. Khalil, S. A. Nada, S. A. Kenawy, K. A. Ahmed, M. M. Salama, M. M. Safar, *Toxicol. Mech. Method*. **2020**, *30,* 620–633. **DOI:**10.1080/15376516.2020.1801926
54. R. S. Ali, W. C. Hmood, M. A. Alsirrag, *Mater. Sci. Eng*. **2019**, *571,* 1–12. **DOI:**10.1088/1757-899X/571/1/012101
55. N. M. Abd-Elrahman, A. S. Farid, K. M. Fararh, *Vet. Med. US*. **2014**, 27, 456–465.
56. S. X. Xinxin, K. A. Dilly, J. Y. Lee, *Mol. Canc. Res*. **2015**, *13,* 1533–1543. **DOI:**10.1158/1541-7786.MCR-15-0237
57. C. G. Vijayameena, M. Subhashini, B. R. Loganayagi, *Int. J. Curr. Microbiol*. **2013**, 2, 1–8.
58. D. Wanders, K. Hobson, X. Ji, *Nutrients*. **2020**, 12, p.684. **DOI:**10.3390/nu12030684
59. F. Wei, J. W. Locasale, *Trend. Cancer*. **2023,** 9, 705–706. **DOI:**10.1016/j.trecan.2023.07.008
60. J. Y. Min, K. S. Chun, D. H. Kim, *Future Oncol*. **2023**, 12, p. 997919. **DOI:**10.3389/fonc.2022.997919
61. C. G. de David, Rodrigues, S. Bona, L. Meurer, J. González-Gallego, M. J. Tuñón, N. P. Marroni, *Toxicol. Pathol*. **2011**, *39,* 949–957. **DOI:**10.1177/0192623311418680
62. T. Akhtar, N. Sheikh, *Toxin Rev.* **2013**, *32,* 43–46. **DOI:**10.3109/15569543.2013.805144
63. L. Antonio, L. B. Rafael, M Roberta, *J. Clin. Pathol*. **2022**, *55,* 641–7. **DOI:**10.1136/jcp.55.9.641
64. M. Domínguez, M. Luján-Hidalgo, D. González-Mendoza, A. Vargas-Díaz, N. Ruiz-Lau, F. Gutiérrez-Miceli, C. Lecona-Guzmán, *Phyton*. **2019**, *88,* p. 139. **DOI:**10.32604/phyton.2019.06546

Povzetek

Rak na jetrih še vedno predstavlja velik globalni zdravstveni izziv, saj njegova pojavnost po vsem svetu narašča. Napovedi kažejo, da bo do leta 2040 število obolelih za rakom na jetrih preseglo milijon. Zato so številni raziskovalci motivirani za izboljšanje terapij in razvoj novih zdravil z minimalnimi stranskimi učinki na zdravje ljudi. Naravni izdelki rastlinskega izvora ponujajo različne farmakološke kemijske strukture in biološko aktivne snovi, ki izkazujejo citotoksične učinke na tumorske celice. Pričujoča študija raziskuje potencialne protirakave lastnosti izvlečka cvetov *Fallopia baldschuanica* proti raku, povzročenemu s tioacetamidom (TAA). Študija je natančno opredelila specifične aminokisline v surovem izvlečku, pri čemer je bil najvišji odstotek metionina, sledili so mu cistein, valin, treonin, tirozin, izolevcin in lizin. Aminokisline so pomembne za različne vidike človekovega zdravja in bolezni. Namen te študije je oceniti potencialni vpliv izvlečka cvetov *Fallopia baldschuanica* na regresijo eksperimentalno povzročenega HCC pri podganah. Ocene so bile izvedene z merjenjem delovanja jeter, ki vključuje aspartatno aminotransferazo, alanin-transaminazo in alkalno fosfatazo. Poleg tega so bili uporabljeni testi antioksidativnih encimov in tumorskih označevalcev ter histopatološki pregledi, ki so potrdili ugotovitve.