Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Research on the effects of the Juncus inflexus subsp. inflexus extract on the liver damage caused by low dose thioacetamide at the rats

Muhittin KAYA
mkaya13@gmail.com
Bitlis Eren University
Emin KAYMAK
Bozok Universitesi
Ali Tuğrul AKİN
Erciyes University
Engin YILMAZ
Bitlis Eren University
Murat KURŞAT
Bitlis Eren University
Birkan YAKAN
Erciyes University

Research Article

Keywords: Juncus inflexus, Caspase-3, Thioacetamide, MDA, GSH, Liver

Posted Date: June 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1716185/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at ChemistrySelect on October 9th, 2024. See the published version at https://doi.org/10.1002/slct.202402981.

Abstract

This study investigated the effects of thioacetimide (TAA) on liver damage by using the extract of the *Juncus inflexus* subsp. *inflexus (*JI) plant. Six experimental groups were formed using 42 8-12-weeks *Sprague dawley* male rats, with seven experimental animals per group. Two doses of normal saline (SF) (i.p) were administered within 120 minutes to each control group. 250 mg/kg single dose of TAA (i.p) was administered 120 minutes after administration of SF in the TAA group. 50 mg/kg single dose of JI extract (i.g) was administered 120 minutes after SF administration to the JI group. TAA+JI group was given 50 mg/kg JI extract 120 minutes after 250 mg/kg TAA was administered. 250 mg/kg of TAA was administered 120 minutes after 50 mg/kg of JI extract was applied to the JI+TAA group. TAA+JI+ group was administered 50 mg/kg JI extract 120 minutes after 250 mg/kg TAA was administered and 2 more 50 mg/kg JI extract were administered after 48 hours. At the end of the trial, blood samples were analyzed for aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) with autoanalyser. Liver tissue sections were examined by staining with hematoxylin eosin (H-E), periodic acid schiff (PAS) and caspase-3. On the other hand, the tissue homogenates were analyzed by Enzyme Linked Immunosorbent Assay (ELISA), in terms of malondialdehyde (MDA) and glutathione (GSH).

In H-E and PAS studies, while a marked damage was detected in TAA group compared to other groups, based on biochemical analysis, only a significant change in parameter AST was determined. Compared to other groups, it was observed that the MDA rose significantly in the TAA group and that the GSH fell and that the expression of the kaspaz-3 was significantly increased.. On the other hand, it was found that lipid peroxidation decreased significantly in the TAA+JI group compared to JI+TAA group.

In conclusion, this study has revealed that the extraction obtained from the *Juncus inflexus* plant has protective and therapeutic properties for the liver.

Introduction

The liver, having critical functions, including elimination of harmful toxic substances, blood making, glucose storage as glycogen, biliary production, defense of the organism, protein and lipid metabolism, and processing of nutrients, is constantly exposed to drugs, environmental pollution, and toxic agents. The liver tries to minimize these effects with its detoxification mechanism, while also repairing the damages it is exposed to (Wang et al., 2005). When the liver becomes inadequate in defense and regeneration, the oxidative stress increases and its natural structure changes (Naziroğlu et al., 1999; Wang et al., 2005) This acute liver damage is a disease accompanied by extensive deterioration of the liver's synthetic, metabolic and detoxiferative functions (Shoeib AM et al., 2015).

Increased reactive oxygen radicals in liver failure caused by drug hepatotoxicity or viral hepatitis are attributed to severe reductions in glutathione level. An increase in proinflammatory cytokines has been observed to result in the activation of nitric oxide and nuclear factor kappa B (NF-KB) (Demure, 2008).

Thioacetamide (TAA) is a hepatotoxic agent, containing carcinogenic and necrogenic compounds (thionosulfur), used for a period in the leather processing, laboratory, textile and paper industries, in addition to extending the shelf life of citrus fruits (Akhtar and Sheikh, 2013; Aycan, 2015). TAA, known since 1948, is responsible for syntholobular necrosis but, when administered in a single dose, it causes acute hepatic toxicity and may lead to the development of chronic cirrhosis or hepatic carcinoma in prolonged exposures (Hajovsky H et al., 2012; Akhtar and Sheik, 2013; El Baz FK et al., 2019). Today, TAA is widely used in the development of empirical liver damage and if given 200 mg/kg, it leads to a reduction in hepatic edema and a decrease in the glycogen amount at the end of the first four hours. At the end of the sixth hour, changes in mitotic activities were observed. One of the damage mechanisms of TAA is the increase in oxidative stress by changing lipid peroxidation, protein oxidation and tissue antioxidant levels (Demir, 2008; Aycan, 2015).

In defense, the enzyme of superoxide dismutase is activated and catalyzes the dismutation of superoxide to H_2O_2. Glutathione peroxidase (GPx) inhibits lipid peroxidation, turns H_2O_2 into water to protect membrane lipids and hemoglobin from oxidative stress. The glutathione disulfide (GSSG) formed in the reactions GPx catalysts is an antioxidant enzyme that converts glutathione (GSH) in the reduction via glutathione transferase (GST). The intracellular level of GSSG increases, which induces an increase in glutathione reductase. Therefore, the rate of GSH-GSSG in cells is of importance (Akşit et al., 2015).

In our study, the rats were used as experimental animal, and as an agent that induces oxidative stress and liver damage, thioacetamide was used and as an agent that is intended to determine its effect, the extract of J*. İnflexus* subsp. *inflexus* plant was used. The plant was collected and extruded by us.

Material And Method

Experimental animals

In this study, 42 male adult *Sprague dawley* type rats, of 8-12 weeks and 150-200 grams, which were raised in the Erciyes University Experimental Research Application and Research Center (DEKAM) (Kayseri, Turkey), were used. The rats which were held in cages were provided with water and nutrients in a 21^{o}C at 12-hours light/dark environment. Experimental groups were formed by weighing the rats and bringing them together so that their weights were close to each other. All of the transactions were carried out in accordance with the International Universal Declaration of Animal Rights.

Plant Material

The plant was collected at an altitude of about 1885 (± 3m) meters at the coordinates 38^{o}26'30" North and 42^{o}22'51" East in the village of Obuz of Tatvan district at Bitlis Province. Plant samples were identified and confirmed by Associate Professor Dr. Murat KURŞAT, who is an instructor at the Biology Department of the Faculty of Arts and Sciences of Bitlis Eren University.

Soxhlet assembly was used to obtain total plant extraction (Tripathi et al., 2014). Previously washed and dried herbal roots were finely chopped and transferred to 500 ml ethanol as 50 grams. After it was boiled at 78^{o}C for 90 minutes, the pulp of the plant was removed and was filtered with extract filter paper. Subsequently, alcohol and essence were separated through the vacuum evaporator. Analyzes were performed by the device SHIMADZU QP2010 ULTRA GC-MS at the Technology Research and Application Center of Erciyes University (Joshi et al., 2016). The components of the extract, which were primarily transformed into gas phase, were separated in gas chromatography, and then the components were assigned according to their mass through mass spectrophotometry.

Before the plant extract was applied to rats, it was thawed in normal saline.

Biological Experiments

The first day of the applications was considered as the first day of the experiment. Six hours after the applications were completed, the livers of the rats were removed by decapiting the blood of the rats by means of intra-cardiac method under the anesthesia of xylazine (10 mg/kg) ketamine (75 mg/kg). Half of the removed livers were taken at -80°C for MDA and GSH and the other half were identified using 10% formaldehyde for histological analyzes. The applications were carried out according to the following plan (Table 1).

Table 1. Distribution of experimental groups and applications.

	Group	*n*	Applications
1	Control	7	Two doses of 0.1 ml normal saline were administered intraperitoneally (i.p.) with 120 minutes intervals.
2	TAA	7	120 minutes after 0.1 ml normal saline was administered intraperitoneally, 250 mg/kg TAA was administered intraperitoneally.
3	JI	7	120 minutes after 0.1 ml normal saline was administered intraperitoneally, 50 mg/kg JI extract was administered via intragastric (i.g.) route.
4	TAA + JI	7	120 minutes after the first 250 mg/kg TAA was given intraperitoneally 50 mg/kg JI extract was administered via intragastric (i.g.) route.
5	JI + TAA	7	120 minutes after the first 50 mg/kg JI extract was given via intragastric (i.g) route, 250 mg/kg TAA was administered intraperitoneally.
6	TAA + JI +	7	120 minutes after the first 250 mg/kg intraperitoneal TAA administration, 50 mg/kg JI extract was administered via intragastric (i.g) route. Only the JI extract, which is the treatment agent, was applied at the same dose for 2 times with 48 hours intervals.
		42	

Biochemical Analysis

Following the experimental applications, blood was taken by means of intracardiac injection for biochemical analysis. The blood was transferred into the gel serum tubes and was centrifuged for 10 minutes for 1500 g per minute and its serums were transferred into eppendorf tubes and kept at +4°C.

The analyzes of the AST, ALT and ALP enzyme levels were performed at the Biochemistry Laboratory of Tatvan State Hospital on MINDRAY BS-2000M automatic biochemical analyser. For the analysis , ALT (Kat. No: ALT1104, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Mindray building, Keji 12th Road South, Hi-tech Industrial Park,Nanshan, Shenzhen, 518057 P.R. China), AST (Kat. No: AST1104, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Mindray building, Keji 12th Road South, Hi-tech Industrial Park,Nanshan, Shenzhen, 518057 P.R. China) ve ALP (Kat. No: ALP1104, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Mindray building, Keji 12th Road South, Hi-tech Industrial Park,Nanshan, Shenzhen, 518057 P.R. China) kits were used.

The liver samples which were kept at -80 °C were homogenized for ELISA analysis of MDA and GSH markers. Tissues were homogenized before study. Then, they were centrifuged and their supernatants were transferred to ependorf tubes in order to be used afterwards. Their amounts were determined as ng/ml at 450 nm by ELISA reader after the relevant transactions were carried out in accordance with the kits of MDA (Cat. No: E0156Ra, Bioassay Technology Laboratory) and GSH (Cat. No: EA0113Ra, Bioassay Technology Laboratory).

Histological Applications

Liver tissues were detected at the end of the experiment in 4 percent formaldehyde solution and they were passed through the increased alcohol series (50 percent, 70 percent, 80 percent, 96 percent, and 100 percent) and dehydration was performed. After the tissues were transparentized with xylene, they were put into paraffin. H-E and PAS were applied to thin sections of 5 µm thickness taken from paraffin blocks, and these sections were covered with closure solution (Entellan®, Merck) and examined under an Olympus BX51 microscope.

Immunohistochemical Applications

Caspase-3 antibodies in liver cells were studied in this study. For this purpose, CASP3 polyclonal antibodies (E-AB-63602; Elabscience, China) were used. Streptavidin-biotin as method, peroxidase as marker and diaminobenzidine tetrachloride (DAB) as chromogen were used. The procedure specified by Öztürk et.al., (2020) was implemented.

Statistical Analysis

For the analysis, Graphpad Prism 9 software was used. One-Way ANOVA was applied for parametric evaluations and Post hoc Tukey test was performed in order to compare the data. The data were determined as mean ± standard deviation. Since the statistical significance level was $p< 0.05$, it was determined as significant.

Results

The Chemical Components of *Juncus inflexus* subsp. *inflexus* Plant

In the specified location, 700 grams of plant root was obtained and decomposed. As a result of the decomposition processes, 30 grams of extract was obtained from the plant roots (Figure 1). 19 peaks were detected as a result of GC-MS analysis. Each peak represents a component. According to the analysis results, the most common components are 10-Methoxy-nb-alpha-methylcorynantheol, as well as ergosterol, anthraquinone and fatty acid derivatives (Table 2.).

Table 2. The percentage of the presence of JI components detected by GC-MS;

Peak	%	Chemical Name
1	0,69	2-Methoxy-4-vinylphenol
2	0,49	Acetamide
3	3,02	Cis-9-Hexadecanoic acid
4	5,14	n-Hexadecanoic acid
5	1,32	Hexadecanoic acid, ethyl ester
6	3,09	9,12-Octadecadienoic acid
7	7,10	Cis-Vaccenic acid
8	1,26	9,12-Octadecadienoic acid, methyl ester
9	2,72	9-Octadecanoic acid, ethyl ester
10	3,78	9-Octadecanoic acid, 2-3dihydroxypropyl
11	2,13	Hexadecanoic acid, ethyl ester
12	1,23	Ergosta-5,7,22-trien-3-ol
13	1,45	9-Octadecanoic acid, 2-3dihydroxypropyl
14	1,58	Ergosta-5,8,22-trien-3-ol
15	3,66	Ethyl iso-allocholate
16	15,71	Ergosterol
17	32,56	10-Methoxy-nb-alpha-methylcorynantheol
18	9,66	Anthraquinone,1,2,4-trimethyl
19	3,41	1,2-Benzendicarboxylic acid, bis(2-ethylhexyl) ester
Total	100	

Biochemical Analyses

In this study, the serum liver enzymes, AST, ALT and ALP levels were examined across groups and no significant difference was determined at the pairwise comparison between groups. Nevertheless, ALT and ALP values in the TAA group were analyzed at a higher level than control group, JI and other groups. The ALT level was measured to be lower in the groups in which TAA was applied before JI than in the group in which JI was applied before TAA. When TAA and TAA+JI+ (P:0.065) and TAA+JI and TAA+JI+ (P:0.053) were compared in terms of AST parameter, no significant difference was determined in the pairwise comparison (Table 3.).

Table 3. The change of serum ALT, AST and ALP levels between groups;

Gruplar	Kontrol	JI	TAA	TAA+JI	JI+TAA	TAA+JI^{+}	*p*
ALT (U/L)	61.44±6.87	63.31±7.55	69.80±10.0	64.75±6.95	68.31±8.39	64.96±10.33	**0.469**
AST (U/L)	110.7±13.2	117.8.0±12.19	121±9.68	122.7±19.05	118.2±13.4	98.2±19.1	**0.041**
ALP (U/L)	371.7±37.14	378.3±72.51	454.7±110.3	370.5±48.73	362.9±19.02	393.7±54.36	**0.105**

The data were expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b and c). P<0.05 was considered as statistically significant.

When the effect of the extract of *inflexus* subsp. *İnflexus plant* on the oxidative stress caused by TAA was examined, the levels of MDA, the end product of lipid peroxidation, as a marker of oxidative damage in the liver caused by free radicals and the levels of free radical deterger endogenous antioxidant were measured and the related data are specified in Table 4. The corresponding data are specified in Table 4. Their quantities were determined in ng/ml at 450 nm wavelength (Table 4.).

Table 4. MDA and GSH levels in liver tissue (ng/ml);

Groups	Control	JI	TAA	TAA+JI	JI+TAA	TAA+JI^{+}	*p*
MDA (ng/ml)	0.67±0.22^{a}	0.66±0.1 2^{a}	1.04±0.27^{b}	0.64±0.21^{a}	0.75±0.13^{ab}	0.78±0.1 1 ^{ab}	0.025
GSH (ng/ml)	605.9±73.11^{ac}	607.4.4±55.17^{ac}	570.4±66.19^{a}	668.9±94.87^{ab}	742.7±40.34^{b}	733.3±45 .89^{bc}	0.0007

The data were expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b and c). P<0.05 was considered as statistically significant.

Histological Findings

At our experiment, in the control group, in terms of all of the H-E staining of liver tissue sections, parenchymal cell chords, having a radial organization from the central vein to the periphery were observed. Hepatic sinusoids were regular between chords and the portal areas were observed to be regular. Nuclei and nucleolus of the cells were observed to be prominent (Figure 2.) and they strongly reacted with PAS (Figure 3.).In TAA group, narrowing was observed in the central vein diameter and in sinusoids close to the central vein. Deterioration of the radial structure of the hepatocyte chords was

observed. It was observed that toxic effects caused cell expansion and degeneration (Figure 2-C.). Anisocytosis was observed due to the expansions in the cells. Examination of liver tissue preparations in the TAA+JI group revealed that there was a limited amount of mononuclear cell infiltration. When the liver tissue preparations of TAA+JI group were examined, a limited amount of mononuclear cell infiltration was observed. It was observed that the liver sections in the JI+TAA group were similar to those of TAA+JI group. After examining the H-E staining of TAA+JI+ group, restructuring of the cell chords was observed. Compared to TAA+JI, group mononuclear cell infiltration was observed to have partially increased.

Immunohistochemical Findings

The effect of TAA on damage to the liver and the extract applied to the damage was evaluated immunohistochemically at the light microscope. When caspase -3 changes from apoptosis effector enzymes were examined, positive cell count increased in all groups by control group (Figure 4.). However, these changes were of statistical significance only between TAA group and other groups (Table 5).

Table 5. The change in the levels of caspase-3 by groups;

GROUPS	Control	JI	TAA	TAA+JI	JI+TAA	TAA+JI+	*p*
Caspase-3	0.63 ± 0.76^{a}	1.20 ± 1.30^{a}	8.66 ± 5.33^{b}	2.20 ± 1.84^{a}	1.50 ± 1.52^{a}	1.33 ± 1.21^{a}	0.0001
The data were expressed as mean ± standard deviation. There is no significant difference between the groups containing the same letter (a, b). P<0.05 was considered as statistically significant.							

Discussion

TAA (C_2H_5NS) is an organosulphur compound and has been used in the leather processing, textile and paper industries (Akhtar and Sheikh, 2013). TAA, known since 1948, can cause centrilobular necrosis, but when is administered as a single dose, it leads to acute hepatic toxicity and can lead to the development of chronic cirrhosis or hepatic carcinoma in prolonged exposures (Hajovsky H et al., 2012; Akhtar and Sheik, 2013; El Baz FK et al., 2019). While TAA is metabolized it is converted to sulfine and sulfene. Sulfine is responsible for the expansion of nucleolus, increase in their nuclear volume, intracellular Ca + concentration and the change in cell permeability, and it inhibits mitochondrial activity. Sulfine is responsible for the release of nitric oxide synthase (NOS), the NF-KB directing to centrilobular necrosis, protein denaturation and lipid peroxidation (Akhtar and Sheikh, 2013; Aycan, 2015: Yahya et al., 2021). As a result of the microscopic examinations made, it has been stated that hepatic edema develops 4 hours after TAA (200 mg/kg) administration, followed by a decrease in glycogen stores, and after about 6 hours, changes in mitotic activity occur and necrosis increases within 24 hours. Subsequently, glycogen stores decreased and mitosis activity changed after approximately six hours. Necrosis increased in 24 hours (Aycan, 2015).

In the studies conducted to establish cystic fibrosis, 200 mg/kg TAA chemical substance was administered intraperitoneally twice a week for three months (Ra et al., 2019). In addition, in the studies conducted, TAA chemical substance was used intravenously at doses of 70 mg/kg and 280 mg/kg in order to generate acute hepatotoxicity. Two doses of 100 mg/kg TAA were administered 24 hours intervals by the studies carried out by Soheib AM et al., 2015, two doses of 100 mg/kg TAA were administered 24 hours intervals by the studies carried out by Aycan (2015) and Zargar et al., (2017) applied a single dose of 300 mg/kg. In another study, in rats administered 200 mg/kg single dose of TAA, measurements performed 6-12-24 hours intervals revealed pulpase-3 in liver and plasma. Caspase-3 activity was determined to be at its highest level in the twelfth hour (Hayami et al., 1999).

In the current study, control group, JI, TAA, TAA + JI, JI + TAA and TAA + JI + groups were established and TAA 250 mg/kg single dose was applied. There were no deaths among rats. 50 mg/kg JI extract (dissolved in normal saline) was

administered as a single dose. In a similar study, the hepatoprotective effect of the *Juncus subulatus* plant was investigated and 50 mg/kg *J. subulatus* extract was administered 3 times per week for 20% ethyl alcohol for 5 weeks (Abdel Razik et al., 2009). In addition, in another study, Juncus species were analyzed for MIC (minimal inhibition concentration) values in comparison with vancomycin. *J. inflexus* was determined to be much more effective than other types (MIC: 9.75• µg/mL (Toth, 2016). Due to the limited studies in the literature on dosing, TAA + JI + was formed as an additional rat group and 50 mg/kg JI extract was administered 120 minutes after 250 mg/kg TAA was administered and 50 mg/kg JI was administered twice 48 hours intervals and a total of 150 mg/kg JI was applied. When the H-E of this group was examined, mild mononuclear cell infiltration was detected. This fact indicates that our 50 mg/kg dose is correct.

The Juncaceae family has been the subject of intense phytochemical studies in recent years. Despite the researches focusing on the Juncaceae family, only eight species of Juncus and Luzula have been extensively studied so far (Bus et al., 2018). There are, however, a very limited number of hepatoprotective studies (Abdel Razik et al., 2009). Plant extracts were obtained from the roots, above-ground organs and seeds, by n-butanol, methanol, n-hexane, water, ethanol and ethyl acetate solvents. Their chemical components were analyzed with GC-MS, HPLC and NMR. (Abdel Razik et al.,2009; Erdem et al., 2015; Atmani et al., 2016; Toth, 2016; Cai et al., 2016; Bus et al., 2018; Stefko et al., 2020). At a study conducted by El Shamy et al., (2015) it was stated that *J. inflexus* plant includes apigenin-4'-O-glucoside, luteolin-5-glucoside, luteolin-4'-O-glucoside, chrysoeriol-7-glucosidesulphate flavinoids In a comprehensive study conducted by Toth (2016), it was determined that this plant contains the following bioactive components: four natural phenanthrene (Jinflexin A, Jinflexin B, Jinflexin C ve Jinflexin D), four dihidrofenantre (juncuenin A, juncuenin B, juncusol, juncuenin D), three phenanthrene (dehidrojuncuenin A, dehidrojunkusol, dehidrojuncuenin B) and flavonoid chrysoeriol.

In our study, *J. inflexus* subsp. *inflexus* plant roots were used. Roots were crushed, separated with ethanol and applied to rats through total intragastric. The analyzes we conducted with the GC-MS revealed predominantly fatty acids and ergosterol anthraquinone derivative in the plant. It has also been reported by Govindappa et al. (2014) and Malathi et al. (2016) that fatty acids also function as antioxidants. The ergosterol in its composition was reported to act as antioxidants by resisting oxidation through in vitro and in silico (molecular modeling) studies (Dupont et al., 2021). In addition, Umanaa et al. (2020) reported the antioxidant activity of ergosterol in their study with the CUPRAC (Cupric Reducing Antioxidant Capacity) test. The antioxidant property of anthraquinone had not been confirmed until recently. However, in a theoretical study conducted, it was revealed that the superoxide anion purification activity of anthraquinone is significantly higher when compared to typical antioxidants such as ascorbic acid and quercetin (Trung et al., 2021). In our study, the total extract of *J. inflexus* plant was applied and its effectiveness was demonstrated experimentally. However, since the components were not applied individually, it could not be determined from which component(s) the efficacy originated.

The enzymes such as ALT and AST are often used as biochemical markers of liver damage. In damaged liver cells, ALT and AST enter the blood circulation and cause an increase in plasma levels. Therefore, an increase in plasma AST and ALT activities in the serum can be considered as an index of damage to liver parenchymal cells (Ra et al., 2019). After the experiments were completed, the *Sprague dawley* type rats used in our experiment were sacrificed after xylazine-ketamine was given, their blood was taken by intracardiac route and their liver enzymes were analyzed. Although there were differences between the groups in the statistical evaluations made as a result of the analyzes, no significance was found in the ALT and ALP parameters ($P < 0.469$ and $P < 0.105$, respectively). Only the change in AST values between the groups was considered as significant ($P < 0.041$), however no significant difference was determined in the pairwise comparison conducted ($P < 0.053$). In a similar study, blood was taken from the retro orbital plexus and biochemical analyzes were performed on the rats, which were sacrificed after five weeks of applications. In this study, significant differences were observed in terms of AST, ALT, ALP, Alb (albumin) and TP (total protein) compared to the alcohol group, control and other groups (Abdel Razik et al., 2009). In another study, while a significant difference was observed between the control group and the other groups in the analyzes conducted after acute liver injury, any change in the AST and ALT parameters of the damage detected in the histological examinations in the TAA + Mel group was not detected (Sayan, 2019).

Liver tissue sections of the control group were observed in their natural structures in the whole H-E staining. Liver sinusoids and chords were regularly organized and portal areas were observed to be regular. The nuclei and nucleolus of the cells were distinct and they reacted strongly with the much-used PAS for general carbohydrate staining. In TAA group, narrowing was observed in vena centralis and sinusoids in general. Chords were determined to be irregular. In addition, there was hepatocyte increase in terms of pyknotic nuclei and eosinophilic cytoplasm. It was determined that the toxic effect also caused enlargement of the cells and increased hydropic degeneration was observed. A weak PAS reaction was detected in the TAA group compared to the control group and JI groups. In a similar study conducted by Abdel Salam et al., (2013), they applied 300 mg/kg TAA and they reported that there was a significant decrease in glycogen stores in the liver tissue sections of the rats. Hepatocyte chords and sinusoids in the TAA + JI group were observed to be more organized compared to the TAA group. It was observed that the histological organization in the TAA + JI + group, to which we administered two doses of JI extract, was closer to its natural structure. However, a small amount of mononuclear cell infiltration was also detected in this group. In a study, the effect of rutin was investigated in the rats administered a single dose of 300 mg/kg TAA, and extensive intracellular vacuolization and infiltration were detected in the TAA group. Also, in this study, it was confirmed histologically that there was a decrease in vacuolization and infiltration with routine administration (Zargar et al., 2017). The histological changes detected in the histological examinations in our study coincide with each other when compared with the findings of MDA and GSH.

In liver damage, MDA, the indicator of lipid peroxidation and GSH, which has an antioxidant effect that prevents oxidative stress and sweeps free radicals have been widely used as two important markers (Seçkin et al., 2008). In a study conducted, the effect of TAA on GSH and MDA was studied in liver tissue homogenate, and it was determined that the level of GSH decreased in the thioacetamide group compared to the control group, and MDA increased (Abdel Salam et al., 2013). In our study, while GSH values were determined to be high in liver tissue homogenates of the groups given JI extract, high MDA and low GSH values were found only in the group given TAA. A significant reduction in MDA in TAA + JI and a significant increase in GSH in TAA + TAA and TAA + JI + groups compared to TAA group has shown that the JI extract relieves TAA pressure on oxidative stress and GSH. In a similar study conducted by Alamri (2019), luteolin and quercetin antioxidants were used instead of JI and it was verified that the pressure of TAA on SOD, CAT and GSH decreased.

The caspases, which are effectors for apoptosis, are caspase-3, caspase-6 and caspase-7 (Seervi and Xue, 2015). Chen et al., (2006) determined in a study that TAA activates p53 and increases caspaz-3. In our study, we investigated the effects of reactive oxygen species elevated by TAA and the *J. İnflexus* subsp. *inflexus* plant on caspase − 3.

According to our immunohistochemical evaluation carried out, an increase in the number of positive cells for caspase-3 was observed in all groups compared to the control group. However, these changes are of statistical significance only between the TAA group and the other groups. The changes in all groups given JI showed similarities with the control group. This verifies the fact that JI extract suppresses the caspase-3 activation caused by TAA. When the preparations were examined microscopically, positive cell locations were usually observed at the lines of the cell surrounding the central vein and in the cells adjacent to it. In addition, the periphery of the classical lobulation was observed. We also verified that mainly nuclei were stained in the intracellular arrangement. This indicates that there is active caspase-3 increase and intracellular location. The studies carried out have reported that caspase-3 is present in the cytoplasm as a pro-enzyme and active caspase-3 passes into the nucleus (Kamada et al., 2005; Schindler et al., 2006; Luo et al., 2010).

As a result, in the chemical component analyzes of the *J. inflexus plant*, it is stated in the literature that the plant contains strong antioxidants, antiviral and antimicrobials, as well as components with antitumor and anti-inflammatory properties. Also, our study has verified the fact that the *J. inflexus* subsp. *inflexus* plant has protective and therapeutic effects on the liver. However, there are very few experimental studied in the literature in this respect. We are of the opinion that our study can be used in the development of treatment tools in liver damage and viral infections, when chemical component analyzes of the organs of the plant are carried out.

Declarations

Acknowledgements: All operations in our study were carried out under the supervision of a veterinarian in accordance with the International Universal Declaration of Animal Rights, with the approval of the Ethics Committee of the Department of Experimental Animals of Erciyes University (Date: 15.05.2019, Decision no: 19/096).

Funding: This work was supported by the Department of Scientific Research Projects of Erciyes University TDK-2019-9295

References

1. Abdel Razik AF, El-Shamy A, Nassar M, et al. Chemical Constituents And Hepatoprotective Activity Of Juncus subulatus. Rev. Latinoamer. Quím. 2009;37(1):70–84.
2. Abdel Salam OM, Mohammed NA, Sleem AA, Farrag AR. The Effect of Antidepressant Drugs on Thioacetamide-Induced Oxidative Stress. Eur Rev Med Pharmacol Sci. 2013; 17(6):735–744.
3. Akhtar T, Sheikh N. An Overview of Thioacetamide-Induced Hepatotoxicity. Toxin Reviews, 2013; 32(3): 43–46.
4. Akşit H, Akşit D, Kara H, Yavuz Ö, Seyrek K, Bildik A. Effects Of N-acetyl Cysteine On Glutathione Metabolism And Lipid Peroxidation In The Experimental Hepatic Intoxication. Ankara Universitesi Veteriner Fakultesi Dergisi. 2015;62(1):1–5.
5. Alamri ZZ. Effect of Luteolin and Quercetin on Thioacetamide Induced Hepatic Fibrosis in Rats. International Journal of Pharmacology, 2019; 15: 863–871.
6. Atmani A, Sekhri L, Khaled B. A comparative study of the antibacterial activity of Juncus maritumus Asch & Buschen; its synergic effect with some standard antibiotics and some other medicinal plants. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2016; 7(6):6–13.
7. Aycan S. Effects of Grape Seed Extract on Thioacetamide-induced Organ Toxicity in Rats. Phd Thesis, Fırat University Faculty of Medicine, Elazig, Turkey 2015.
8. Bus C, Toth BE, Stefko D, Hohmann J, Vasas A. Family Juncaceae: Promising Source of Biologically Active Natural Phenanthrenes. Phytochem Rev. 2018; 17: 833–851.
9. Cai Y, Qiu R, Lu Y, et al. Hypoglycemic Activity of Two Anthraquinone Derivatives From Juncus setchuensis Buchen. Int J Clin Exp Med, 2016; 9(10):19664–19672.
10. Chen LH, Hsu CY, Weng CF. Involvement of P53 and Bax/Bad triggering apoptosis in thioacetamide-induced hepatic epithelial cells. World J Gastroenterol. 2006; 12(32): 5175–5181.
11. Demir K. Investigation of the Efficacy of Kefır in the Prevention of Thioacetamide-Induced Acute Liver Failure in Rats. Phd Thesis, Süleyman Demirel University Faculty of Medicine, Isparta, Turkey 2008.
12. Dupont S, Lessard PF, Cruz RG, Lafarge C, Grangeteau C. Antioxidant Properties of Ergosterol and Its Role in Yeast Resistance to Oxidation. Antioxidants, 2021; 10: 1024.
13. El-Baz FK, Salama A, Salama RAA. Therapeutic Effect of Dunaliella salina Microalgae on Thioacetamide- (TAA-) Induced Hepatic Liver Fibrosis in Rats: Role of TGF-β and MMP9. BioMed Research International, 2019; 2019:1–9.
14. El-Shamy A, Abdel-Razek A, Nassar M. Review: Phytochemical review of Juncus L. genus (Fam. Juncaceae). Arabian Journal Of Chemistry. 2015;8:614–623.
15. Erdem F, Cetinkaya N, Nisbet C, Altin E. Estimation of organic matter digestibility, metabolizable energy, phenolic compounds and antioxidant activity of stems and seeds of the Juncus acutus plant in ruminant nutrition. South African Journal Of Animal Science. 2015;45(5):502–509.
16. Govindappa M, Prathap S, Vinay V, Channabasava R. Chemical Composition of Methanol Extract of Endophytic Fungi, Alternarsa Sp. of Tebebuia argentea and Their Antimicrobial and Antioxidant Activity. International Journal of Biological & Pharmaceutical Research. 2014; 5(11): 861–869.

17. Hajovsky H, Hu G, Koen Y, et al. Metabolism and Toxicity of Thioacetamide and Thioacetamide S-Oxide in Rat Hepatocytes. Chemical Research in Toxicology, 2012; 25(9): 1955–1963.
18. Hayami S, Ikeda K, Suna F, Tanaka K, Kojo S. Increase of caspase-3 activity in rat liver and plasma by thioacetamide. Biochemical pharmacology, 1999; 58(12):1941–1943.
19. Joshi S, Rai N, Kumar N. Gas Chromatography-Mass Spectroscopy Analysis of Phytochemical Constituents of Methanolic Extract of Needles of Pinus wallichiana. 2016; 9(2):55–57.
20. Kamada S, Kikkawa U, Tsujimoto Y, Hunter Y. Nuclear Translocation of Caspase-3 Is Dependent on Its Proteolytic Activation and Recognition of a Substrate-like Protein(s). Journal of Biological Chemstry, 2005; 280(2):857–860.
21. Luo M, Lu Z, Sun H, et al. Nuclear entry of active caspase-3 is facilitated by its p3-recognition-based specific cleavage activity. Cell Research, 2010; 20: 211–222.
22. Malathi K, Anbarasu A, Ramaiah S. Ethyl Iso-allocholate from a Medicinal Rice Karungkavuni Inhibits Dihydropteroate Synthase in Escherichia coli: A Molecular Docking and Dynamics Study. Indian J Pharm Sci 2016;78(6):780–788.
23. Naziroglu M, Çay M, Üstündag B, et al. Protective Effect of Vitamin E on Carbon Tetrachloride-Induced Liver Damage in Rats. Cell Biochemistry and Function 1999; 17:253–259.
24. Öztürk E, Kaymak E, Akin AT, Karabulut D, Ünsal HM, Yakan B. Thymoquinone is a protective agent that reduces the negative effects of doxorubicin in rat testis. Hum Exp Toxicol. 2020;39(10):1364–1373.
25. Ra SH, Shin RH, Ri HC, et al. Effect of Lesimarin Against Thioacetamide-Induced Liver Cirrhosis in Rat. Braz. J. Pharm. Sci., 2019; 55:1–9.
26. Sayan M. Effect of Melatonin on Hepatocellular Cell Death in Thioacetamide-Induced Acute Liver Injury. Master Thesis, Erciyes University, Institute of Health Sciences, Kayseri, Turkey 2019.
27. Schindler CK, Pearson EG, Bonner HP, et al. Caspase-3 Cleavage and Nuclear Localization of Caspase-Activated DNase in Human Temporal Lobe Epilepsy. Journal of Cerebral Blood Flow & Metabolism, 2006; 26(4):583–589.
28. Seçkin Ş, Alsancak Ş, Başaran Küçükergin C, Aydın M. Effect of D-Galactosamine on Oxidative Stress and Apoptosis in The Liver of Rats. İst Tıp Fak Derg, 2008; 71:29–32.
29. Seervi M, Xue D. Mitochondrial Cell Death Pathways in Caenorhabiditis elegans. Curr Top Dev Biol. 2015;114:43–65.
30. Shoeib AM, Said E, Nader MA, Salem HA, Ammar EM. Tiron Mitigates Thioacetamide-Induced Acute Liver Injury. J.Food Pharm.Sci. 2015; 3(3):55–63.
31. Stefko D, Kusz N, Barta A, et al. Gerardiins A–L and Structurally Related Phenanthrenes from the Halophyte Plant Juncus gerardii and Their Cytotoxicity against Triple-Negative Breast Cancer Cells. Journal of Natural Products, 2020; 83(10): 3058–3068.
32. Toth BE. Bioactive Secondary Metabolites From Juncaceae Species. PhD Thesis, University Of Szeged, Faculty Of Pharmacy, Szeged (Hungary) 2016.
33. Tripathi B, Bhatia R, Pandey A, Gaur J, Chawala G, Walia S, Choi EH, Attri P. Potential Antioxidant Anthraquinones Isolated from Rheum emodi Showing Nematicidal Activity Against Meloidogyne incognita. 2014; 4: 1–9.
34. Trung NQ, Thong NM, Cuong DH, et al. Radical Scavenging Activity of Natural Anthraquinones: a Theoretical Insight. ACS Omega, 2021; 6:13391–13397.
35. Umanaa M, Eima V, Garaub C, Rossellóa C, Simal S. Ultrasound-Assisted Extraction of Ergosterol and Antioxidant Components from Mushroom By-Products and The Attainment of A B-Glucan Rich Residue. Food Chemstry, 2020; 332: 127390.
36. Wang H, Weit W, Wang NP, et al. Melatonin Ameliorates Carbon Tetrachloride- Induced Hepatic Fibrogenesis In Rats Via Inhibition Of Oxidative stres. Life Sciences 2005; 77:1902–1915.
37. Yahya SMM, Shalaby RH, Mannaa FA, et al. Hepatoprotective Effects of Chitosan on ThioacetamideInduced Liver Toxicity in Male Albino Rats. Biointerface Research in Applied Chemstry, 2021; 11(6): 14490–14505.

38. Zargar S, Wani TA, Alamro AA, Ganaie MA. Amelioration of Thioacetamide-Induced Liver Toxicity in Wistar Rats by Rutin. Int J Immunopathol Pharmacol. 2017; 30(3): 207–214.

Figures



Figure 1

Juncus inflexus subsp. *inflexus* plant (at its location).

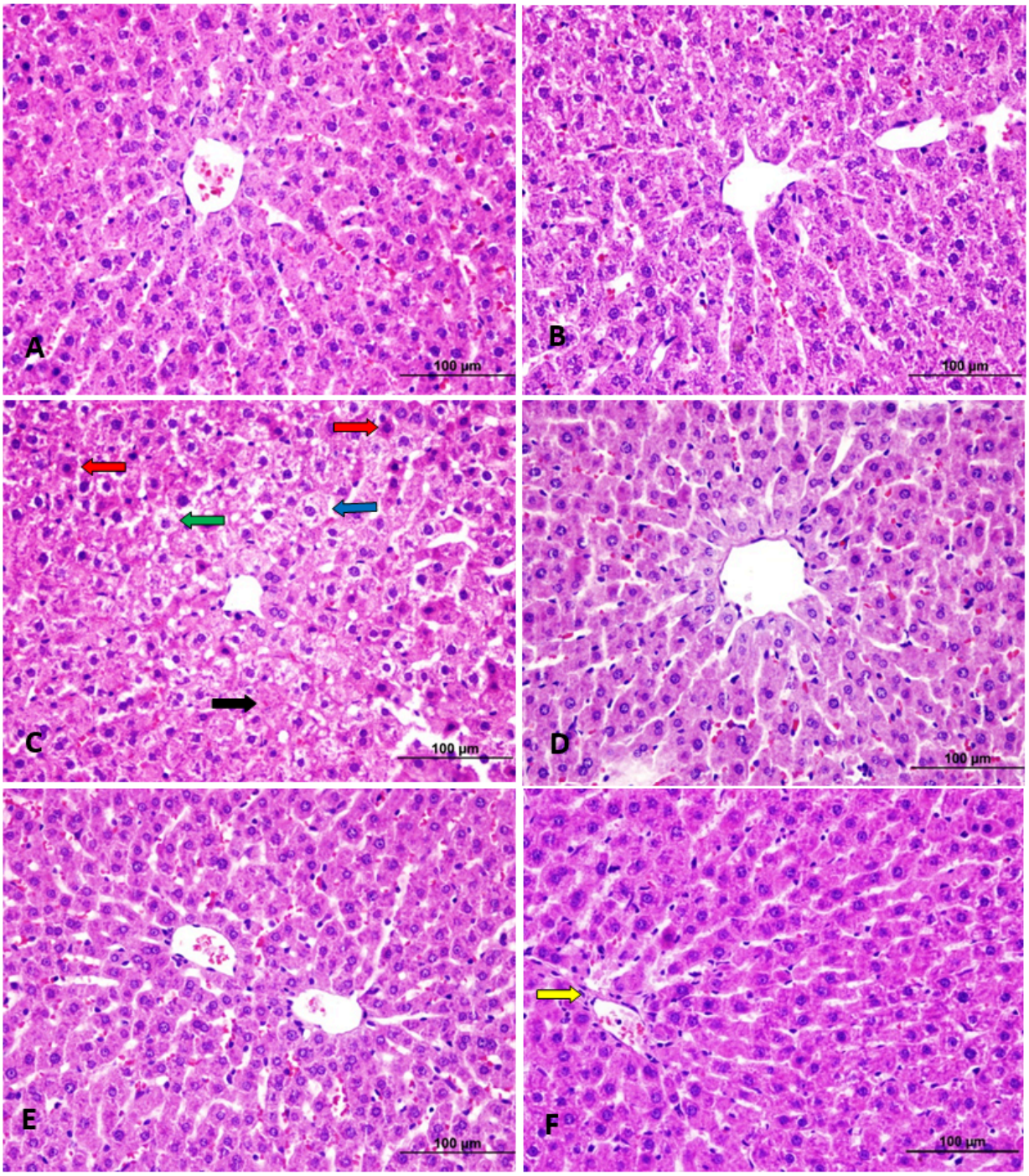


Figure 2

H-E staining of groups, 40X magnification. A) Control group, B) JI group, C) TAA group, D) TAA+JI group, E) JI+TAA group, F) TAA+JI+ group. Red arrow: eosinophilia, green arrow: hydropic degeneration, yellow arrow: infiltration, blue arrow: cellular expansion, black arrow: necrotic area.

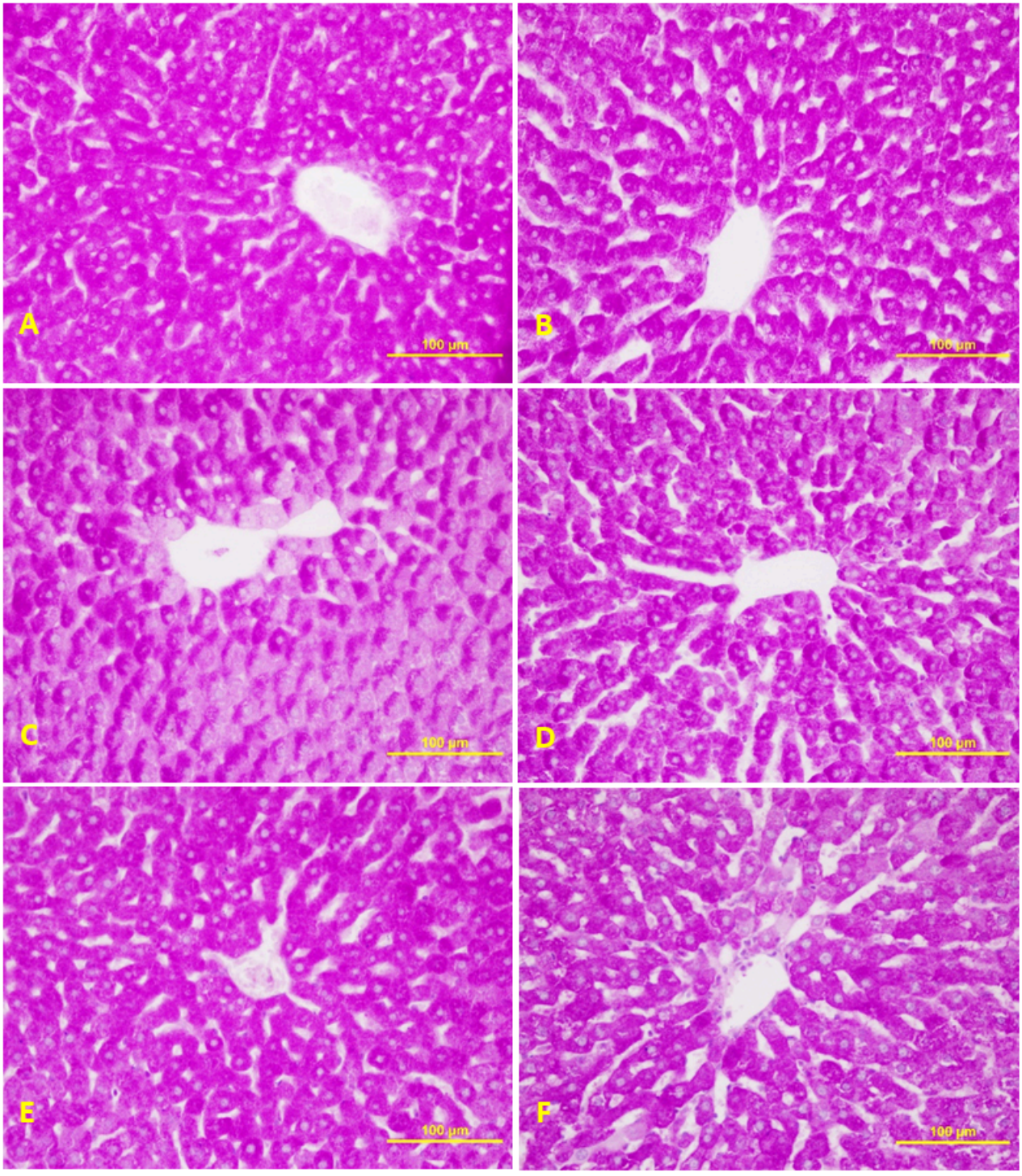


Figure 3

PAS Reaction of the Groups, 40X magnification. A) Control group, B) JI group, C) TAA group, D) TAA+JI group, E) JI+TAA group, F) TAA+JI+ group.

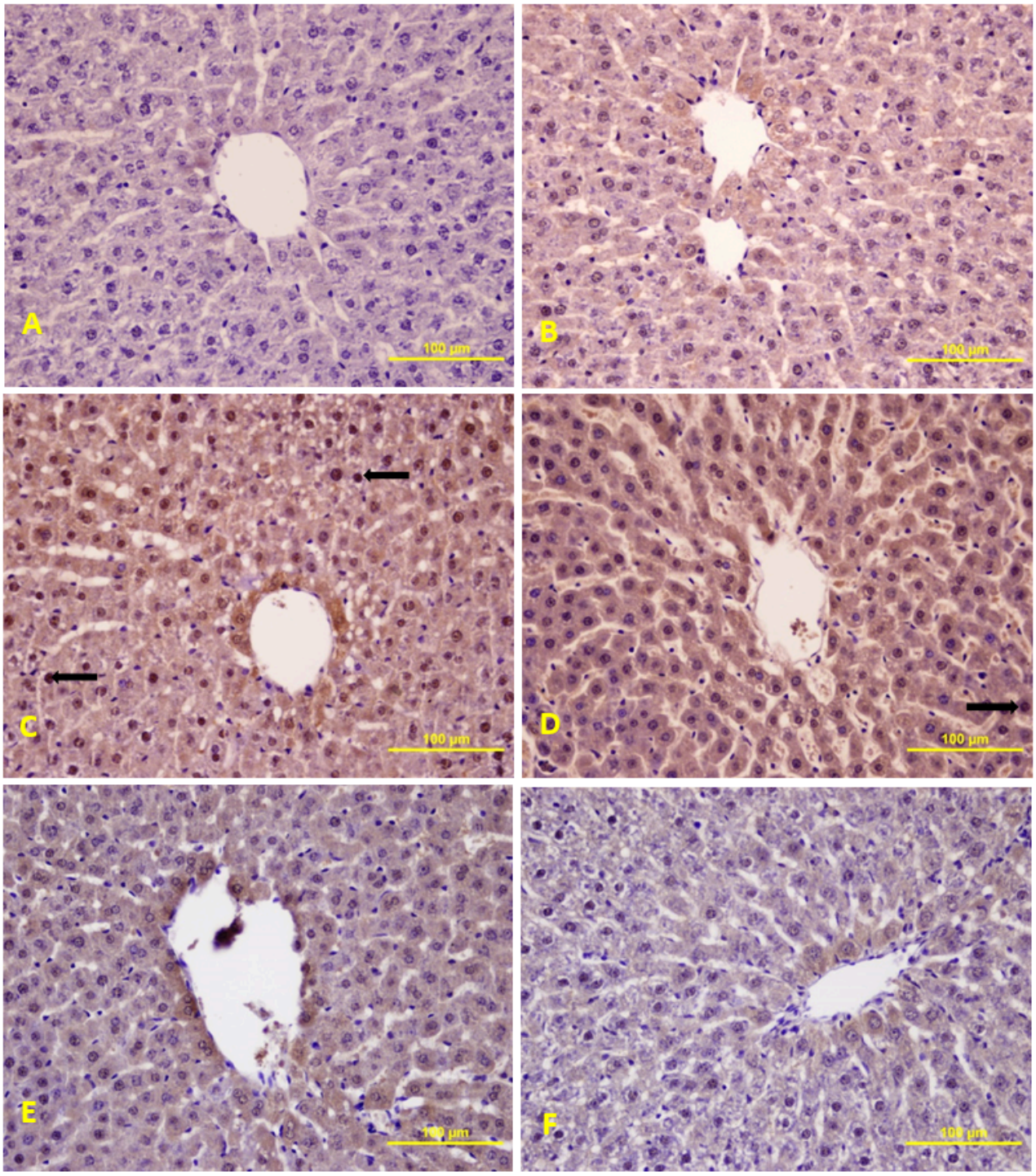


Figure 4

Caspase-3 stained with Immunohistochemical technique, 40X magnification. A) Control group, B) JI group, C) TAA group, D) TAA+JI group, E) JI+TAA group, F) TAA+JI+ group. Black arrow: CASP3+ cells.