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BIOCHEMICAL, HEMATOLOGICAL AND HISTOPATHOLOGICAL EVALUATION OF THE FOOD-SAFETY OF THE LEAF EXTRACT OF PAPAVER SOMNIFERUM IN RATS

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ABSTRACT

The fresh shoots of Papaver somniferum L. (opium poppy) are used as a flavouring by the local population in the regions where it is grown. The aim of this research was to determine the food-safety of the extract obtained from the fresh leaves of Papaver somniferum L. plant.

The extract of the leaves of the opium poppy plant, were dissolved in 1 ml of drinking water and administered to healthy male rats, in 4 different doses (400, 800, 1600 and 2000 mg/kg body weight/day) on a single day to evaluate the acute and each day for 30 days to evaluate the subacute effect. Blood was withdrawn for hematological and biochemical analysis. Lung, heart, liver and kidney tissues were taken for pathological examination.

It was observed that the ALT enzyme decreased at the dose of 800, 1600 and 2000 mg/kg body weight/day; furthermore, AST, GGT and CK-MB enzymes decreased at the doses of 1600 and 2000 mg/kg body weight/day in the subacute phase ($p<0.05$).

It can be stated that the water extract obtained from the leaves of Papaver somniferum L. has no negative effect on the biochemical, hematological and pathological parameters in the given doses, furthermore the extract may have a protective effect on the liver tissue.

KEYWORDS:
Food-safety, papaver somniferum, leaf, serum chemistry, hepato-protective

INTRODUCTION

The development of the opium poppy plant from the Papaveraceae family encompasses stages such as seed, shoot, rosette, branch formation, flowering, seed development and capsule formation (growing stage), seed and capsule maturity (maturation) [1]. It is known that the fruits of the plants of this family, which occur in the type of trees or shrubs, are very common and that seeds are contained in capsules and hairy leaves [2].

In different parts of the Papaver somniferum L. plant, substances with different properties appear. While alkaloids are more excessive in the seed and capsule, phenolic substances are more intense in the shoots and leaves. Furthermore from those phenolic substances trans-P-coumaric acid, myricetin, caffeic acid and quartzine are known to have remarkable antioxidative effects.

Papaver somniferum L. is an area of interest in research studies, mainly because as it is an industrial product made for its alkaloids in the capsule and the oil in the seed. In particular, the chemical content of the seeds, seed capsule and flowers of this plant have been in focus of several studies [3,4]. The fresh shoots of Papaver somniferum L. are also known to be used as a flavouring by the local population in regions, where it is grown [5,6]. On the other hand, there are no studies on the food-safety in the sprout of this plant. Research on toxication has particularly focused on analysis during the encapsulation time where the amount of alkaloid increases [7,8].

In this context the aim of this study was to evaluate the food-safety of fresh Papaver somniferum L. plant extract in sprout, which is the period where it is consumed as food, also to provide further scientific analysis for its various uses in the future.

MATERIALS AND METHODS

Plant material. Freshly sprouted (rosette stage) Papaver somniferum L. (opium poppy) plant, planted in autumn for human consumption, reaching a length of 10 cm, was used in the study. The leaves of the plant were picked off from the stalk, washed with distilled water and dried under room conditions in the shade.

Preparation of the plant extract. Dried Papaver somniferum L. leaves were grounded in a household rondo into powder. The powdered samples (20 g) were extracted with a 400 ml solvent in the Soxhlet device for 2 hours on average at boiling temperature [9]. Then, the extracts were filtered through filter paper, and the solvents were evaporated at boiling temperature in the evaporator [10]. Further drying was done using a freeze-drying system (FreeZone plus, LABCONCO, USA) at -84ºC for 24 hours, after which the extract was dried for another 48 hours in the oven. The extract was then stored at 4°C. At the same time, with the lyophilized samples used in this study, the dissertation entitled "In vitro determination of the effect of Papaver somniferum L. leaf extract on small intestinal contractions" was completed [11].

Animals and experimental design. This study was carried out at the Research Center of Afyon Kocatepe University/Turkey following the approval from Afyon Kocatepe University Ethics Committee (AKÜHADYEK-303-13). A total of 70 healthy male rats, aged 3-4 months, obtained from the Research Center were held in cages, 7 rats in each cage, in a well ventilated room, at a room temperature of 25°C and at a 12 hours' day/night cycle light adjustment. Two groups were formed, an 'acute group' (n=35) and a 'subacute group' (n=35), in order to evaluate the acute effect and subacute effect of lyophilized Papaver somniferum L. extract. Each group including a control group was subdivided into 5 subgroups and the extract was dissolved in 4 different doses (400, 800, 1600 and 2000 mg/kg body weight/day) into 1 ml of drinking water and administered by gavage. Blood was withdrawn under general anesthesia for biochemical and hematological analysis from the hearts of the rats, 1 hour after the extract was given to the acute group. Whereas in the subacute group the extract was given once daily for 30 days and on the 30th day blood was withdrawn under general anesthesia from the hearts of the rats, for biochemical and hematological analysis. Lung, heart, liver and kidney samples have been taken after the blood withdrawal from both groups and stored in formaldehyde for pathological examination.

Body weight recording. Rats were weighed at day 1 in the acute, subacute phase groups, and on each day for 30 days in the subacute phase group.

Hematological analysis. Red and white blood cell count from blood was determined manually by using a hemocytometer, which was taken to tubes with EDTA. The number of hemoglobin was determined by Drabkin's cyanomethemoglobin method, hematocrit by microhematocrit method. Lymphocyte, neutrophil, monocyte, eosinophil and basophil percentage ratios were calculated from blood smears stained with May Grünwald-Giemsa [12].

Biochemical analysis. The biochemical analysis were performed with commercial kits (Abbott Diagnostics, Chicago, IL, USA) using an autoanalyzer (Abbott C8200 autoanalyzer; Chicago, IL, USA) for the parameters alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), creatine phosphokinase-myocardial binding factor (CK-MB), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), high density lipoprotein (HDL), low density lipoprotein (LDL), amylase, lipase , albumin, cholesterol, total protein (TP) and calcium.

Pathological examination. Lung, heart, liver and kidney samples were stored in a buffered neutral 10% formaldehyde solution after dissection. Forty-eight hours later, these organs were cut into smaller pieces and passed through increasing concentrations of alcohol and xylene, and blocked in paraffin wax. From these blocks 4-5 microns thick cuts have been made with a microtome and the sections were stained with Hematoxylin-Eosin (HE) technique. The Sections were examined under a light microscope.

Statistical analysis. SPSS 13.0 statistical package software, was used for the statistical analysis. Statistical comparisons were made by "Variance analysis" and "Tukey" test for amplitude values obtained separately for each tissue. P values less than 0.05 were accepted as the level of significance. Results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

This study was carried out to determine the food-safety of fresh Papaver somniferum L. leaf plant extract in rats. The selected dose range was between 400 and 2000 mg/kg body weight/day, as there is no previous scientific data about the food-safety of Papaver somniferum L. leaf extract.

No death was observed in the acute group of this study, but two deaths were observed in the subacute group, one in the control group and one in the group where Papaver somniferum L. leaf extract was administered at a concentration of 1600 mg/kg. If the entire study is taken into account, both deaths can be interpreted as being of natural cause and does not relate to the administration of Papaver somniferum L..

In this study, the administration of the extract to rats in the subacute group did not result in a statistically significant difference in body weight ($p>0.05$), but in the groups in which the extract was administered at a dose of 1600 and 2000 mg/kg body weight/day, a body weight reduction of approximately 10% was observed (Table 1).

TABLE 1
The subacute effect of Papaver somniferum L. leaf extract administered in various doses on body weight in rats.

	Control	400 mg/kg	800 mg/kg	1600 mg/kg	2000 mg/kg	P
Body weight (g) 1st day	161,00±2,22	163,66±3,35	162,50±3,10	162,50±2,82	163,16±1,77	0,967
Body weight (g) 30th day	196,50±2,90	193,66±3,83	188,33±6,18	183,33±2,29	181,50±4,47	0,077

Groups where the Papaver somniferum L. leaf extract are administered orally at doses of 400- 2000 mg/kg for 30 days. Values are given as mean ± SD for a group of seven animals each.

TABLE 2
The acute and subacute effect on hematological parameters, of Papaver somniferum L. leaf extract administered in various doses (n: 7).

Parameters	Group	Control	400 mg/kg	800 mg/kg	1600 mg/kg	2000 mg/kg	P
Red Blood Cell ($x10^{12}$/L)	Acute	6,89±0,14	7,10±0,28	7,01±0,17	6,97±0,23	7,12±0,20	0,936
	Subacute	7,22±0,12	7,55±0,14	7,24±0,12	7,52±0,79	7,36±0,16	0,257
Hemoglobin (g/L)	Acute	13,09±0,30	13,06±0,24	13,04±0,33	13,12±0,18	13,41±0,23	0,849
	Subacute	12,94±0,19	12,83±0,26	12,73±0,15	12,52±0,35	12,85±0,26	0,620
Hematocrit (%)	Acute	45,25±0,88	44,12±0,93	45,22±1,06	44,85±0,93	46,37±1,29	0,661
	Subacute	45,75±0,70	45,25±0,52	46,55±0,92	45,42±0,68	44,38±1,33	0,775
White Blood Cell ($x10^{12}$/L)	Acute	3,52±0,14	3,64±0,13	3,76±0,25	3,54±0,18	3,58±0,15	0,888
	Subacute	3,91±0,19	4,04±0,17	4,10±0,23	4,00±0,25	3,92±0,17	0,956
Lymphocyte (%)	Acute	54,37±0,70	57,75±1,68	57,00±1,49	57,14±1,26	56,62±1,26	0,457
	Subacute	53,37±0,56	56,12±1,48	57,00±0,88	55,57±0,75	54,25±1,30	0,124
Neutrophil (%)	Acute	40,62±0,99	37,25±1,27	37,33±1,42	38,85±1,22	39,87±1,21	0,229
	Subacute	40,75±0,72	37,87±1,44	37,66±1,45	39,57±1,49	41,87±1,57	0,152
Eosinophil (%)	Acute	2,75±0,25	2,12±0,22	2,66±0,28	2,42±0,29	2,12±0,29	0,339
	Subacute	2,87±0,22	2,37±0,26	3,00±0,33	2,85±0,34	2,25±0,36	0,334
Monocyte (%)	Acute	2,25±0,313	1,87±0,295	1,88±0,200	1,57±0,368	2,25±0,365	0,518
	Subacute	2,37±0,32	1,87±0,29	2,22±0,27	1,85±0,40	2,37±0,37	0,657
Basophil (%)	Acute	0,250±0,163	0,375±0,182	0,444±0,242	0,142±0,142	0,250±0,163	0,813
	Subacute	0,25±0,16	0,12±0,12	0,55±0,17	0,28±0,18	0,25±0,16	0,452
Mean Corpsucular Volume (fl)	Acute	65,94±2,23	62,67±2,38	64,72±1,76	64,71±2,38	65,56±2,96	0,884
	Subacute	63,46±1,36	59,97±1,02	64,39±1,78	60,40±1,07	63,39±2,93	0,323
Mean Corpuscular Heomoglobin (pg)	Acute	19,05±0,57	18,66±1,03	18,72±0,69	18,95±0,88	18,93±0,62	0,996
	Subacute	17,95±0,30	17,01±0,42	17,62±0,42	16,67±0,58	17,51±0,47	0,311
Mean Corpuscular Hemoglobin Concentration (%)	Acute	290,32±10,14	297,42±10,75	289,88±10,11	293,13±9,45	290,65±8,65	0,981
	Subacute	283,53±6,51	284,17±8,37	274,20±4,60	275,92±7,81	278,65±9,42	0,880

Groups where the Papaver somniferum L. leaf extract are administered orally at doses of 400-2000 mg/kg for 30 days. Values are given as mean ± SD for a group of seven animals each.
In the study, no significant differences were seen between the acute and subacute phase group (P>0.05).

TABLE 3
The acute and subacute effect on biochemical parameters of Papaver somniferum L. leaf extract administered in various doses (n: 7).

Parameters	Group	Control	400 mg/kg	800 mg/kg	1600 mg/kg	2000 mg/kg	P
Urea mg/dl	Acute	58,00±1,625	62,00±2,345	62,75±1,14	63,50±1,89	64,50±2,81	0.228
	Subacute	63,25±1,49	63,87±1,25	64,75±1,09	68,25±1,43	68,12±1,84	0,057
Blood Urea Nitrogen	Acute	27,06±0,758	28,93±1,094	29,28±0,532	29,63±0,882	30,10±1,311	0,228
	Subacute	29,51±0,695	29,80±0,583	30,21±0,508	28,10±0,667	31,79±0,858	0,057
Creatin mg/dl	Acute	0,422±0,007	0,426±0,020	0,406±0,007	0,430±0,005	0,421±0,009	0,646
	Subacute	0,408±0,004	0,395±0,005	0,420±0,008	0,410±0,004	0,408±0,010	0,316
Cholesterol mg/dl	Acute	73,62±1,20	71,37±2,26	70,12±3,56	74,50±1,401	72,87±1,84	0,649
	Subacute	74,00±3,41	66,83±5,79	76,00±2,00	68,14±3,54	63,12±3,12	0,113
Triglycerides mg/dl	Acute	38,37±1,30	35,87±1,23	38,25±1,013	39,50±1,45	36,00±0,70	0,141
	Subacute	39,50±1,94	35,50±1,20	38,00±1,77	32,42±4,71	33,75±2,28	0,394
High-density lipoprotein. mg/dl	Acute	49,50±1,79	44,00±1,67	44,62±2,99	48,75±1,44	46,50±1,26	0,201
	Subacute	49,50±1,80	46,83±3,70	49,66±1,45	47,00±3,01	42,25±2,56	0,269
Low-density lipoprotein. mg/dl	Acute	14,00±0,70	13,37±1,19	13,50±0,86	14,00±0,75	14,25±0,86	0,921
	Subacute	12,50±1,17	12,16±1,19	14,00±0,89	12,285±0,94	11,62±0,65	0,507
Total Protein g/dl	Acute	5,85±0,068	5,68±0,131	5,70±0,134	5,78±0,104	5,65±0,122	0,715
	Subacute	5,80±0,089	5,63±0,190	5,88±0,147	5,82±0,080	5,33±0,229	0,131
Albumin g/dl	Acute	3,10±0,046	3,05±0,077	3,07±0,031	3,17±0,031	3,10±0,059	0,536
	Subacute	3,13±0,071	3,01±0,047	3,15±0,061	3,05±0,052	2,98±0,063	0,411
Aspartate aminotransferase U/L	Acute	167,25±4,19	152,75±9,15	158,75±6,02	159,00±4,39	165,50±13,27	0,729
	Subacute	176,66±25,14a	161,66±9,16a	172,33±9,86a	129,00±5,94b	123,75±11,72b	0,020 *
Alanine aminotransferase U/L	Acute	44,50±1,11	45,37±1,19	51,25±2,55	49,25±3,24	49,25±2,83	0,230
	Subacute	51,50±3,52a	48,83±3,08ab	42,66±2,07b	40,42±2,73b	43,00±1,92b	0,037 *
Gamma-glutamyl transferase U/L	Acute	3,75±0,250	3,75±0,163	4,00±0,46	3,25±0,163	3,25±0,313	0,344
	Subacute	3,50±0,22ab	4,00±0,44a	2,83±0,16bc	2,85±0,40bc	2,37±0,26c	0,011 *
Alkaline phosphatase U/l	Acute	236,50±14,91	275,75±16,53	273,25±8,28	251,50±16,17	228,50±22,34	0,172
	Subacute	220,16±11,71	276,83±11,74	239,00±24,08	248,85±17,86	244,62±11,83	0,228
Creatine phosphokinase-myocardial binding factor U/L	Acute	1442,5±93,73	1212,2±83,70	1494,2±86,49	1283,0±135,27	1328,2±154,10	0,411
	Subacute	1391,8±237,35a	1382,6±128,80a	1419,8±167,57a	942,1±54,48b	675,7±48,96b	0,011 *
Lipase U/L	Acute	5,00±0,422	4,75±0,365	4,62±0,375	5,00±0,267	5,62±0,595	0,507
	Subacute	5,33±0,49	4,33±0,210	4,83±0,47	5,714±0,35	4,375±0,419	0,088
Glucose mg/dl	Acute	142,75±3,94	159,75±7,18	163,50±5,80	154,75±7,23	152,25±8,34	0,251
	Subacute	148,16±7,23b	168,50±5,70b	149,00±8,11b	157,57±7,81b	173,00±15,00a	0,118
Calcium mg/dl	Acute	10,02±0,066	10,11±0,24	10,07±0,137	9,81±0,199	10,17±0,070	0,577
	Subacute	9,97±,070	9,96±0,33	9,80±0,16	10,11±0,15	9,90±0,18	0,849

Groups where the Papaver somniferum L. leaf extract are administered orally at doses of 400-2000 mg/kg for 30 days. Values are given as mean ± SD for a group of seven animals each. (*p <0.05)

[11] States that lyophilized extracts obtained from Papaver somniferum L. leaves increase the contraction of the small intestine at the dose of 1 mg/ml, possibly through calcium channels. In this context, it is thought that the Papaver somniferum L. extract used at dose of 1600 mg / kg and above reduces the utilization of nutrients by decreasing the time of stay of the intestinal content in the intestines, thus creating a negative effect on body weight.

In this study, the administration Papaver somniferum L. leaf extract in the acute and subacute groups did not cause a change in hematological parameters ($p>0.05$; Table 2). The hematopoietic system and in particular bone marrow is one of the most sensitive tissues for toxic substances and is an important index of physiological and pathological status in humans and animals [13,14]. In toxicity studies, analysis of blood parameters in animal trials are considered to have a very high predictive value for toxicities in the human hematological system [13,15]. This shows that Papaver somniferum L. extract has no negative effects on the hematopoietic system at the doses used in the study.

There was no difference in biochemical parameters in the acute group of the study ($p>0.05$; Table 3). However, AST and ALT enzymes decreased at the administration of 1600 and 2000 mg/kg body weight/day and GGT enzyme decreased at the administration of 2000 mg/kg body weight/day of Papaver somniferum L. leaf extract in the subacute group ($p<0.05$; Table 3). Since many enzymes in serum are tissue specific, increases in these enzymes are used to diagnose necrosis and degeneration caused by diseases in liver, heart, kidney and skeletal muscle tissues. Although AST is found in high concentrations in skeletal muscle, heart muscle, liver, kidney, brain and red blood cells, it is an indicator of liver and muscle damage [16,17].

Alanine aminotransferase, formerly known as serum glutamic pyruvic transaminase, is found mainly in the liver, and in small amounts in kidney, muscle tissue and other organs. After digestion of food, it catalyses in the liver nutrients to energy. This enzyme is located in liver cells under normal conditions and can also be found in small amounts in the blood circulation due to the dying liver cells. Elevated levels of the enzyme in the blood circulation are accepted as an indicator of damaged hepatocytes [18]. While a significant increase in serum ALT concentration in cattle and sheep is observed in active cardiomyopathy cases; it is mostly considered as a liver-specific enzyme in humans, dogs, cats, rats and rabbits [19]. In hepatocytes, AST is found to be 80% in mitochondria, 20% in cytosol, while ALT is found only in the cytosol.

The enzyme GGT is found in liver and kidneys. Elevations in serum of this enzyme have been associated with liver diseases. These increases are observed especially in cases of long-term chronic liver damage [20], hepatobiliary obstruction and hepatocellular necrosis [18]. The ALP enzyme, which is involved in removing phosphate groups from various molecules, including nucleotides, proteins and alkaloids, is located in bone, liver and the intestinal wall [20]. Serum concentrations of the enzyme increase in cases of hepatobiliar obstruction, decreased biliary secretion, bone diseases and neoplasia [18]. The use of medicinal plants in the treatment of liver diseases, are mainly used either to protect the liver or to support the treatment, thereby hepatocytes are regenerated or their functions are facilitated [21].

The phenolic substances in the composition of these plant extracts also have protective effects on the liver [22]. Quercetin [23] and kaempferol [24] are just two of the phenolic substances commonly found in the plant kingdom. While the Papaver somniferum L. leaf extract used in this study contained 94.56 mg/g phenolic compound, quercetin and kaempferol in the water extract of this plant are the dominant phenolic components obtained from this extraction. In this context, our research findings show that the administration of Papaver somniferum L. leaf extract at high doses such as 1600 mg/kg and 2000 mg/kg body weight/day does not cause any harm to the liver, and has in addition a possible protective effect. It is believed that this protective effect is caused by the phenolic substances in their structure.

In this study, it was determined that 1600 and 2000 mg/kg body weight/day Papaver somniferum L. leaf extract decreased serum CK-MB enzyme level similar to GGT (liver enzymes) ($p<0.05$; Table 3). The enzyme CK has three isoforms (CK1/CK-BB; CK2/CK-MB; CK3/CK-MM), which have high activity in skeletal and cardiac muscle [25]. CK-MM occurs in skeletal and cardiac muscle, CK-BB in brain and epithelial tissues, CK-MB in many tissues, but in particular in the cardiac muscle where the activity is highest after skeletal muscles. CK-MB is elevated in acute myocardial infarction in humans and chronic cardiomyopathies in animals [26]. Within this context the findings of our research show that high doses of Papaver somniferum L. leaf extract have a protective effect on the heart without causing muscle degeneration.

[11] Reported that Papaver somniferum L. leaf extract contains a significant level of phenolic substance, the amount and content of the phenol substance of Papaver somniferum L. varies depending on the type of solvent, accordingly the ethanol extract contains the highest amount of phenolic substance, while this is lowest in the water extract. Since the extract obtained with ethanol is rich in phenol content, it further increases the antioxidant activity [27]. It is known that quercetin and kaempferol are the most common found phenolic substances in water extracts. Such phenolic substances with strong antioxidant activity have a protective effect on tissues [28]. In this context, it is considered that the

protective effect of Papaver somniferum L. leaf extract on the liver and heart in the subacute group can be attributed to the high content of phenolic substances contained in this extract. In this study it was determined that the Papaver somniferum L. leaf extract did not change the total protein, albumin, Na, Creatinine and BUN in the acute as well as in the subacute groups ($p<0.05$; Table 3).

Serum Na, Creatinine, and BUN levels are the most commonly assessed parameters to evaluate kidney function [29]. These parameters provide information about the nephrotoxic effect and the level of intracellular, extracellular fluid and acid-base metabolism. Total protein and albumin levels vary depending on liver and kidney damage and extracellular fluid volume [29, 30]. In this regard, the results of the research show that Papaver somniferum L. leaf extract does not cause nephrotoxic effects at the doses used in the study.

In the present study, it was observed that Papaver somniferum L. leaf extract had no influence on the serum lipid profile (cholesterol, triglyceride,

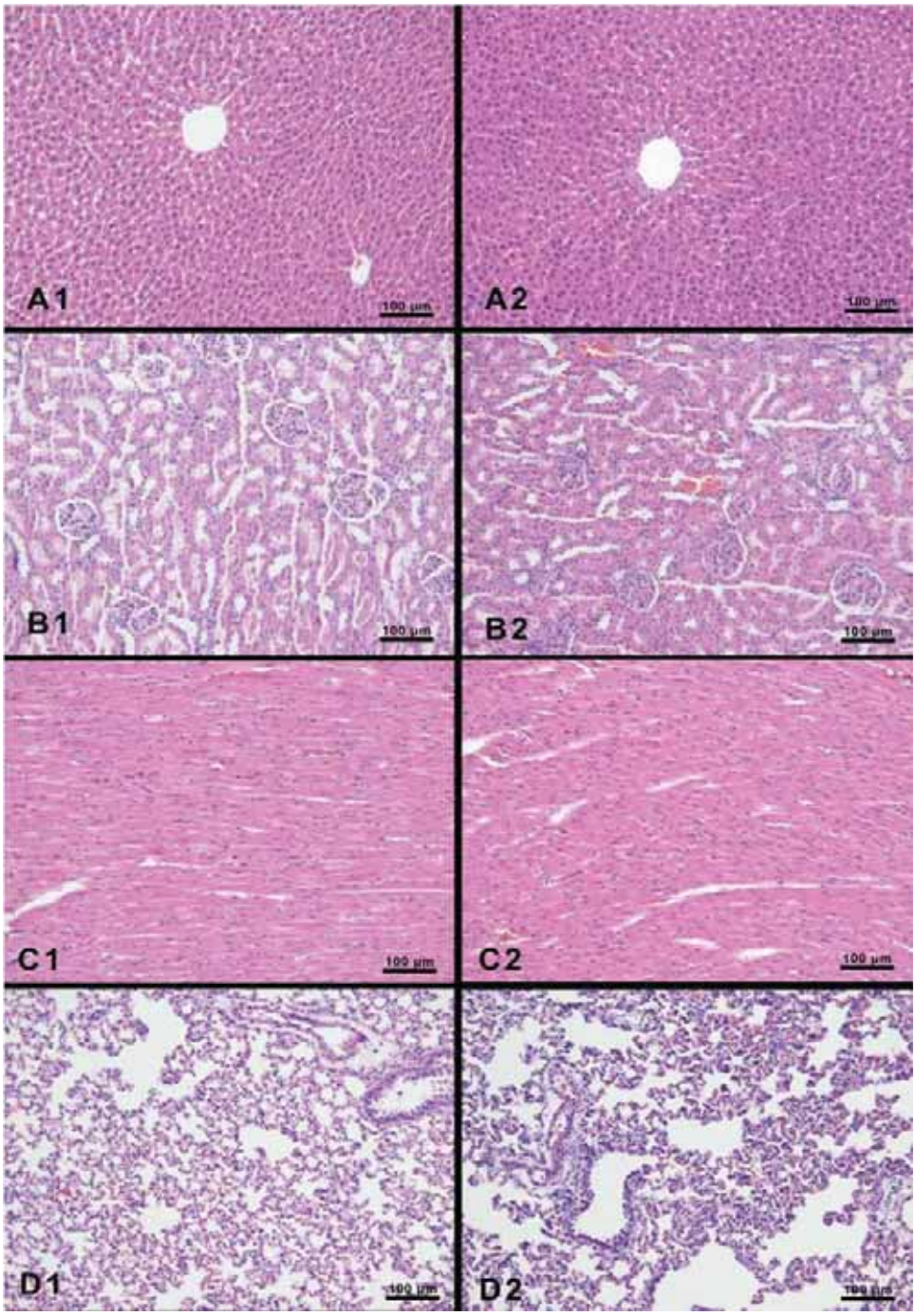

FIGURE 1
Histopathological appearance of tissues belonging to the subacute groups, Liver (A1-A2), Kidney (B1-B2), Heart (C1-C2), Lung (D1-D2). Normal appearance in all organs

HDL cholesterol, LDL cholesterol) in the acute and subacute groups (p> 0.05; Table 3). Cholesterol biosynthesis is mainly carried out by the reductase enzyme 3-hydroxy 3-methylglutaryl-coenzyme A (HMG-CoA), which regulates the mevalonate pathway in the liver [31]. This enzyme is considered to be a therapeutic target for lowering blood cholesterol. It has been reported that many herbal extracts can block cholesterol synthesis through the rate-limiting step in cholesterol biosynthesis in the human body by competitive inhibition of the HMG-CoA enzyme [31, 32]. [32] Reported that the difference in the solvent used in the extraction is important for the effect of herbal extracts on the HMG-CoA enzyme, and in this context the ethanol/water or chloroform extraction is more effective than the water extraction.

The absence of pathological changes (Figure 1) in liver, heart, lung and kidney tissues were found to be consistent with the hematological and biochemical findings.

CONCLUSION

The use of water extract from the leaves of Papaver somniferum L. which is also consumed as human food, has shown in our studies that it has no toxic effect in the given doses. On the contrary, it can be assumed that it has a protective effect on the liver, which is crucial for regulating the functioning of various physiological processes.

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