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Toxicity study of methanol extract of *Rosa damascena trigintipetala* in mice

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Abstract

Rose is one of the most important ornamental plants, with economic and cultural importance. In this work, the methanol extract of Taif rose (*Rosa damascena trigintipetala* Dieck) was investigated for its toxicity (Acute, Sub-chronic and Chronic) and long-term side effects. In addition, the effect of dose accumulation on physiological parameters and histopathological changes in normal mice was studied. The results of this study showed that no toxicity indication of methanol extract of Taif rose during acute, sub-chronic and chronic periods on physiological parameters while the pathological studies showed minimal changes in the vital organs. From this work, it is clear that Taif rose methanol extract could be utilised as a good natural, affordable, and safe therapy.

Keywords: Taif Rose; methanol extract; acute; sub-chronic; chronic toxicity; mice

INTRODUCTION

Historically, natural products derived from plants and animals have been the source of virtually all medicinal preparations [1]. Using natural products increase daily for the discovery of new medicinal agents. Natural products from plants are the richest natural sources of drugs and continue to be used in pharmaceutical preparations. Many active ingredients have been discovered in plants and used directly as patented drugs like Artemisinin and Taxol[2, 3]. Due to the significant increase in worldwide consumption of herbal medicine, its safety and toxicity of it have been highlighted.

Rosa species (Family Rosacea) is one of the most fundamental ornamental crops with an important economic, cultural, and symbolic value. It is referred to as the flower king [4, 5]. *Rosa damascena* is one of the most important Rosa species whose products (rose concrete, rose water, rose oil, rose absolute, and dried petals have long been used in cosmetics, perfumes, medicinal purposes, and food industries [6,7]. Rose products in addition to their perfuming effects was reported to possess a wide range of pharmacological activities in folkloric medicine such as hypnotic, analgesic, antispasmodic, anticonvulsant, anti-inflammatory, anti-oxidants, and anti-microbial activities [5,8-11]. *Rosa damascena trigintipetala* Dieck (Taif rose) is one of the most important commercially propagated rose in Taif, Saudi Arabia. Our previous many reports on Taif rose (essential oil, concrete and absolute oils, organic solvents extracts, and by-product) revealed the importance and value of this plant [3, 10, 12-16].

It is known that acute and chronic liver damage, which causes high morbidity as well as mortality worldwide, is induced by a variety of pathophysiological conditions. Liver toxicity has increased due to exposure to high levels of environmental toxins [17].

Previous research by the same authors [13] studied the acute, sub-chronic and chronic toxicity of water by-product extract of Taif rose in adult Swiss albino mice reported that Taif rose water by-product extract exhibited excellent safety profile in acute, sub-chronic and chronic toxicity studies in mice, the pathological examinations of the internal organs revealed no pathological abnormality and the LD_{50} was > 6000 mg/kg.

In this work, the safety and side effects of Taif rose 80% methanol extract were investigated in

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experimental animals (mice) by using large doses for a long period of time and the effect of dose accumulation on physiological parameters and histopathological changes.

MATERIALS AND METHODS

Chemicals

All solvents, standards and reagents were of high quality from Sigma-Aldrich Chemicals. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA).

Preparation of Extract:

200 grams of dried Taif rose powder were immersed in 1.5 L of 80% MeOH and left for one week with shaking for one hour daily. At the beginning of the next week, the extraction process was started for three times day by day. The solvent was removed under vacuum using rotary evaporator affording known weight of dried methanol extract (62.3 g).

Animals:

Laboratory outbred Swiss albino mice (CD-1), weighing 22±2 g were used; they were obtained from the Schistosome Biology Supply Center (SBPC), Theodor Bilharz Research Institute (TBRI) Giza, Egypt. Mice were caged and maintained on a standard commercial pelleted diet (El-Kahira company for oils and soap, Egypt), with free access to food and water ad libitum. They were housed in controlled laboratory conditions at constant temperature (25 ± 2 °C) and humidity (50 ± 15%) under 12 h light/dark cycles. All the animal procedures were conducted according to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health. All efforts were taken to treat the mice humanely, to follow ethical guidelines, and to employ only the number of animals required to yield acceptable scientific data.

Acute toxicity (LD_{50}):

The acute toxicity of methanol extract (80%) of Taif rose was studied using 42 adult normal Swiss albino mice. Mice were sub-divided into seven subgroups, each group contain six mice. All subgroups were treated orally with rising doses of 500, 1000, 2000, 3000, 4000, 5000 and 6000 mg/kg of 80% methanol extract of Taif rose. Mortality rates were recorded 24 hrs post treatment. The LD_{50} (The lethal dose that killed 50 % of the animals) was determined using computerized program "PCS" (Pharmacologic calculation system).

Sub-chronic toxicity:

A group of adult normal Swiss albino mice (25 mice) were used to study the sub-chronic toxicity of 80% methanol extract of Taif rose. The mice were separated into two subgroups: normal (10 mice) and treated (15 mice). Five % of LD_{50} of 80% methanol extract of Taif rose was used daily for 28 days. Animals body weight was recorded before as well as every week during drug administration and at the end of durations. Mortality rates were recorded during the durations. Animals were sacrificed 24 hrs after the end of treatment, vital organs were weighted, histopathological changes were examined, and liver and kidney functions were also tested [13].

Chronic toxicity:

A group of adult normal Swiss albino mice (48 mice) was used to study the chronic toxicity of 80% methanol extract of Taif rose. Mice were subdivided into two subgroups, normal group contain 23 mice and treated group contain 25 mice. At the start of the experiment, 5% of the LD_{50} of an 80% methanol extract of Taif rose was employed for two days, and on the third day, the dose was increased by 5% of the LD_{50} for three months. Mortality rates were recorded during the durations. Every month, a group of mice was sacrificed; total body weight and vital organs were weighted, histopathological changes were examined and liver and kidney functions were also examined [13].

Parameters of assessment:

a)-Biochemical parameters.

I-Liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assayed spectrophotometrically using the commercially available kits according to the methods of Reitman and Frankel, [18]; Kind and King, [19].

II-Kidney function tests: blood urea and creatinine were assayed spectrophotometrically using the commercially available kits according to the method of Henry, [20].

b) - Histopathological studies.

Specimens from the liver, kidneys, heart, lungs, intestine and spleen were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes and Masson's trichrome stain for collagen fibers[21] and then examined microscopically.

Statistical Analysis

Data are expressed as Mean ± SEM. One-way ANOVA test followed by LSD-post hoc test was performed to determine the significance difference among the mean values of different groups using SPSS, software package version 16.0 (Chicago, IL, USA).

RESULTS AND DISCUSSION

Toxicological studies:

Acute toxicity

No death in adult Swiss albino mice (0 % mortality) were observed 24 hrs post treatment with rising doses of 80% methanol extract of Taif rose starting from 500 mg/kg up to 6000 mg/kg body weight orally. The results of acute toxicity study revealed that the $LD_{50}$was > 6000 mg/kg this indicate that the extract is safe in Swiss albino mice at this dose. Previous research by the same authors [13] on water by-product extract of Taif rose found that in an acute toxicity study, no death in adult Swiss albino mice (0 % mortality) were observed 24 hours after oral administration of increasing doses of water by-product extract of Taif rose ranging from 500 mg/kg to 6000 mg/kg body weight, and the LD_{50} was > 6000 mg/kg.

Sub-chronic and chronic toxicity

In the sub-chronic and chronic toxicity studies, several parameters were thoroughly investigated. When compared to the normal control group, the body weights and vital organ weights of mice treated with a 80% methanol extract of Taif rose remained unchanged during the sub-chronic (28-day) and chronic (three-month) treatment periods (Tables 1- 3). No mortality was observed during sub-chronic toxicity period.

In chronic toxicity, the percentage mortality of an 80 % methanol extract of Taif rose was 12% compared to 9% in the control group (three versus two mice) at the first month, and 20% compared to 7% in the control group (three versus one mouse) at the second month. There was no death in the third month (Table 4). No significant differences were recorded in total body weight and vital organs weight of 80% methanol extract during three months compared to the control animal group during this period (Tables 5- 6). The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the methanolextracts. Serum ALT, AST, and ALP levels are among the most important laboratory indicators of liver tissue injury[22].The levels of liver enzymes (ALT, AST, and ALP) and renal enzymes (blood urea and creatinine) in the 80 % methanol extract treated group did not differ significantly from the normal control group (Table 7).

Previous study by the same authors [13] on water by-product extract of Taif rose showed that, in Sub-chronic toxicity, no mortality was observed during sub-chronic toxicity period and there were no significant differences in total body weight, vital organ weight, and liver (ALT, AST, and ALP) and kidney (blood urea and creatinine) function tests of water by-product extract over three months when compared to the normal animal group. In chronic toxicity, the percentage mortality of water by-product extract of Taif rose was similar at 1st month, where the percentage mortality was 12.5% compared to 6.25% in normal group at the 2nd month and the 3rd month showed no mortality, also no significant differences were recorded in total body weight, vital organs weight and Liver (ALT, AST and ALP) and kidney (blood urea and creatinine) enzymes tested of water by-product extract during three months compared to the normal animal group during this period.

These findings show that the 80 % methanol extract of Taif rose had no toxic effect during the sub-chronic and chronic toxicity periods, with a low proportion of death during the second month of the chronic toxicity phase, which is often the first evidence of toxicity [23].

Table (1): Total body weight of 80% MeoH extract of Taif rose (Sub chronic toxicity)

Total body weight					
Animal groups	**Start**	**1st week**	**2nd week**	**3rd week**	**4th week**
Normal N=10	22.74±0.87	22.70±0.89	24.74±0.98	24.32±0.89	23.53±0.88
80% MeoH ext. N=15	21.79±0.47	22.04±0.59	23.21±0.72	23.00±0.75	23.02±0.74

N=number of animals in each group Values presented are means ± SEM.

Table (2): Vital organs weight of 80% MeoH extract of Taif rose (Sub chronic toxicity)

Vital organs weight						
Animal groups	**Liver**	**Lung**	**Heart**	**Spleen**	**Kidneys**	**Intestine**
Normal N=10	1.00±0.04	0.15±0.01	0.11±0.01	0.10±0.01	0.27±0.02	1.06±0.06
80% MeoH ext. N=15	1.02±0.04	0.14±0.01	0.12±0.01	0.08±0.01	0.27±0.02	1.28±0.07

N=number of animals in each group Values presented are means ± SEM.

Table (3): Liver and kidney function tests of 80% MeoH extract of Taif rose (Sub chronic toxicity).

Liver and kidney function tests					
Animal groups	**ALT U/L**	**AST U/L**	**ALP IU/L**	**Urea mg/dL**	**Creatinine mg/dL**
Normal N=10	27.00±2.19	123.30±7.94	57.30±6.60	37.52±2.82	1.81±0.11
80% MeoH ext. N=15	28.10±1.98	127.03±7.53	59.00±4.81	38.09±1.70	1.90±0.10

N=number of animals in each group Values presented are means ± SEM.

Table (4): Percentage mortality of 80% MeoH extract of Taif rose during three months.

Percentage mortality			
Animal groups	**1st month**	**2nd month**	**3rd month**
Normal	2/23 (9%)	1/15 (7%)	0/7 (0%)
80% MeoH ext.	3/25 (12%)	3/15 (20%)	0/6 (0%)

Table (5): Total body weight of 80% MeoH extract of Taif rose during three months.

Total body weight				
Animal groups	**Start**	**1st month**	**2nd month**	**3rd month**
Normal	21.64±0.85 N=23	23.24±0.91 N=21	26.20±0.99 N=14	29.11±0.72 N=7
80% MeoH ext.	22.22±0.95 N=25	23.91±1.21 N=21	26.44±1.38 N=12	30.46±1.85 N=6

N=number of animals in each group. Values presented are means ± SEM.

Table (6): Total body weight and vital organs weight of 80% MeoH extract of Taif rose during three months.

Total body weight and vital organs weight (g)								
Times	**Animal groups**	**Total body weight**	**Liver**	**Lung**	**Heart**	**Spleen**	**Kidneys**	**Intestine**
1st month	**Normal N=6**	21.58±0.75	1.00±0.07	0.20±0.01	0.12±0.01	0.11±0.02	0.24±0.03	1.81±0.15
	80% MeoH ext. N=7	20.53±1.41	1.06±0.09	0.22±0.02	0.12±0.02	0.08±0.01	0.22±0.02	2.12±0.16
2nd month	**Normal N=7**	24.27±1.77	1.06±0.09	0.18±0.01	0.16±0.02	0.08±0.01	0.33±0.03	1.36±0.10
	80% MeoH ext. N=6	23.02±0.73	1.40±0.10	0.21±0.02	0.10±0.01	0.12±0.03	0.30±0.02	1.61±0.08
3rd month	**Normal N=7**	29.21±0.72	1.34±0.06	0.21±0.01	0.17±0.01	0.12±0.01	0.44±0.02	1.41±0.03
	80% MeoH ext. N=6	30.56±1.85	1.62±0.19	0.26±0.05	0.17±0.01	0.10±0.02	0.42±0.03	1.66±0.07

N=number of animals in each group. Values presented are means ± SEM.

Table (7): Liver and kidney function tests of 80% MeoH extract of Taif rose during three months.

Liver and kidney function tests						
Times	**Animal groups**	**ALT U/L**	**AST U/L**	**ALP IU/L**	**Urea mg/dL**	**Creatinine mg/dL**
1st month	**Normal N=6**	20.17±0.75	37.36±0.83	100.17±16.32	58.18±2.77	1.86±0.12
	80% MeoH ext. N=7	21.00±1.25	36.30±1.91	102.74±6.51	54.40±3.87	2.00±0.10
2nd month	**Normal N=7**	26.67±0.62	119.71±7.19	100.91±10.78	41.05±2.05	1.77±0.08
	80% MeoH ext. N=6	30.86±2.02	117.00±9.17	95.18±9.07	43.10±1.80	1.90±0.06
3rd month	**Normal N=7**	43.20±8.70	134.33±6.09	76.00±5.23	59.40±4.40	1.24±0.13
	80% MeoH ext. N=6	52.30±7.31	133.36±5.53	70.58±6.43	60.44±5.29	1.12±0.23

N=number of animals in each group Values presented are means ± SEM.

Pathological Findings

Normal control.

The examined organs (Liver, intestine, heart, lungs, kidneys and spleen) were normal. The **liver** showed normal hepatocyte and sinusoidal architectures, blue stained nuclei of varied shapes and numbers (one or sometimes two for each cells) and portal areas. Branches of portal vein, hepatic artery and bile duct were visualized in these portal areas. The **kidneys** showed normal glomerular tufts and renal tubules. These tubules were varied from the proximal, distal and collecting tubules, which mostly lined with cuboidal epithelium. The **heart** showed normal pericardium, myocardium and endocardium. The coronary blood vessels and the myocytes of myocardium were in normal pattern. The **lungs** revealed normal bronchi, bronchioles and alveolar spaces. The interlobular and interalveolar septa were thin and in normal pattern. The **intestine** revealed intact mucosa that lined with columnar epithelium and few scattered goblet cells, sub-mucosa and lamina propria. The **spleen** showed normal lymphoid aggregation in the white pulp and sinusoids in the red pulp besides normal splenic capsule and trabeculae (Fig 1).

Sub-chronic toxicity.

The examined organs of 80% methanol extract of Rose, were normal except for some reversible changes were reported. The **liver** showed normal hepatic architecture and central vein besides rare vacuolar degeneration in the cytoplasm of some hepatocytes. The **kidneys** revealed normal glomerular and tubular structure and slightly congested glomeruli. The **heart** showed normal cardiac myocytes. The **lungs** showed bronchioles and alveoli. The **intestine** with mucinous degeneration and slight increased numbers of the goblet cells and without leukocyte infiltrates. The **spleen** was normal (Fig 2).

Chronic toxicity.

1st month

The lesions of 80% methanol extract were almost similar to those described with the sub-chronic toxicity. The **liver** showed mild hydropic degeneration, slight congestion and few lymphocytes in the portal area. The **kidneys** revealed congestion of renal blood vessels. The **heart** showed slight congestion in coronary blood vessel with mild perivascular edema. The **lungs** revealed slight thickening in the interalveolar septa with mild alveolar collapse around the bronchioles and alveolar ducts. The **intestine** showed mild mucinous degeneration in the lining epithelium and slight activation or metaplasia of Paneth cells in the crypts of Lieberkuhn. The **spleen** was normal white pulp and slightly congested red (Fig 3).

2nd month

The **liver** of 80% methanol extract showed mild hydropic degeneration and slight congestion of the hepatic blood vessels. The **kidneys** revealed mild vacuolations and hydropic degeneration in the tubular epithelia and normal glomeruli. However, the kidneys showed hemorrhage beneath the renal pelvis and in the renal medulla. The **heart** showed granular eosinophilic sarcoplasm and slightly congested coronary blood vessels. The **lungs** revealed peribronchiolar aggregations of round cells with thickening the adjacent interalveolar septa with few lymphocytes infiltration. The **intestine** showed edema and few lymphocytes infiltration in the sub-mucosa, mild mucinous degeneration in the lining epithelium. The **spleen** was normal (Fig 4).

3rd month

The **liver** showed moderate hydropic degeneration and round cells infiltrations in the portal areas. The other portal areas revealed dark brown pigments of hemosiderosis and fibroblast proliferation infiltrated with few mononuclears. Moderate hyperplasia in the lining epithelium of bile ducts encircled with few fibrous connective tissues was noticed. The fibrous tissue around the hyperplastic bile ducts stained light blue by Masson's trichrome stain. The **kidneys** showed round cells aggregation at corticomedullary junction and slight vacuolations in the renal tubular epithelium. Cystic dilation of some renal tubules was observed with eosinophilic material and lined by flattened epithelia with no evidence of glomerular damages. The **heart** showed focal irregular areas of Zenker's necrosis and hemorrhage. The **lungs** revealed peribronchiolar congestion of blood vessels, hemorrhage and lymphocytes infiltrations besides perivascular edema. Focal inflammation in the alveolar septa, characterized by the infiltration of inflammatory cells, mononuclear cells and lymphocytes in the pulmonary parenchyma, was observed with mild alveolar collapse. The **intestine** showed sub-mucosal round cells infiltration and rarely necrosis in the intestinal villi. Mucinous degeneration was also detected. The spleen revealed numerous megakaryocytes and hemosiderosis of brown pigments in the red pulp. Meanwhile the white pulp was normal (Fig 5&6).

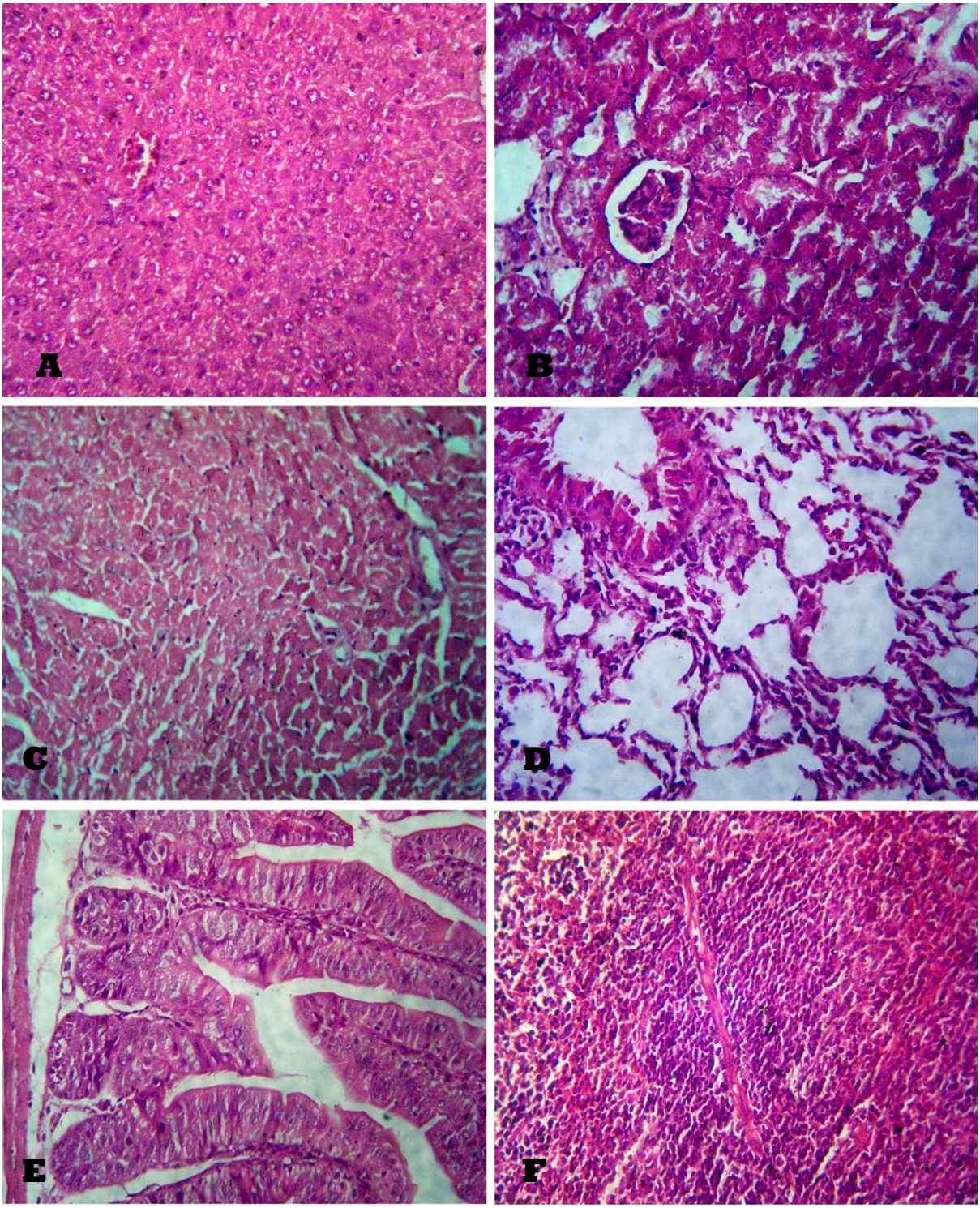

Fig. (1): Normal control group shows: liver with normal hepatocyte and sinusoidal architectures (A), kidney with normal glomerular tufts and renal tubules (B), heart with normal myocytes of myocardium and blood vessels (C), lung with normal bronchi, bronchioles and alveolar spaces (D), intestine with intact mucosa (E) and spleen with normal white and red pulps (F). **HE x 400**.

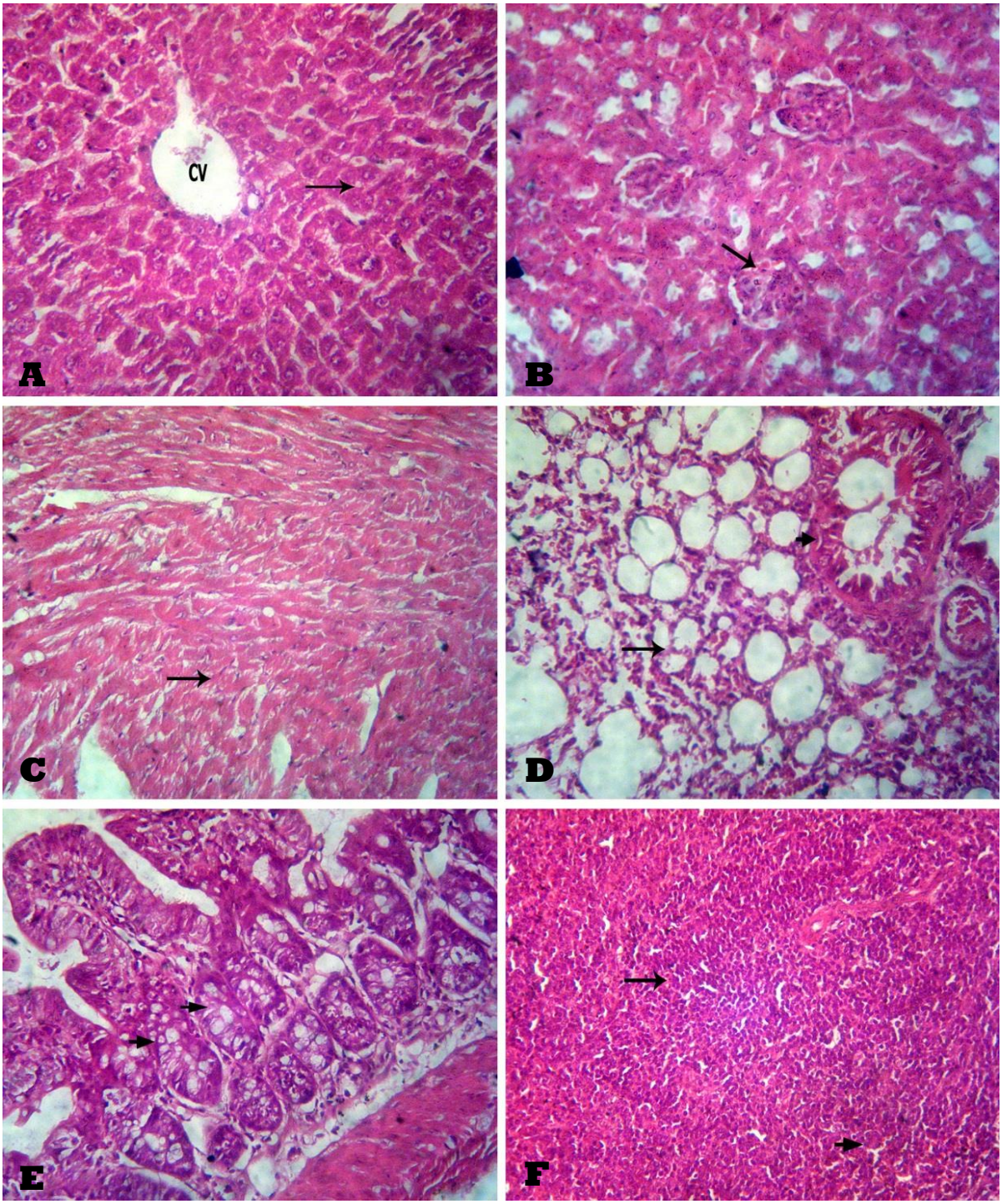

Fig. (2):Sub-chronic toxicity of methanol extract group shows: liver with normal hepatic architecture and central vein (CV) besides rare vacuolar degeneration in the cytoplasm of some hepatocytes (arrow) (A), kidney with normal glomerular and tubular structure, slightly congested glomeruli (arrow) (B), heart with normal cardiac myocytes (arrow) (C), lung with normal bronchiole (arrow head) and alveoli (arrow) (D), intestine with mucinous degeneration and slight increased numbers of the goblet cells (arrow heads) and without leukocyte infiltrates (E) and spleen with normal white (arrow) and red (arrow head) pulps (F). **HE x 400**.

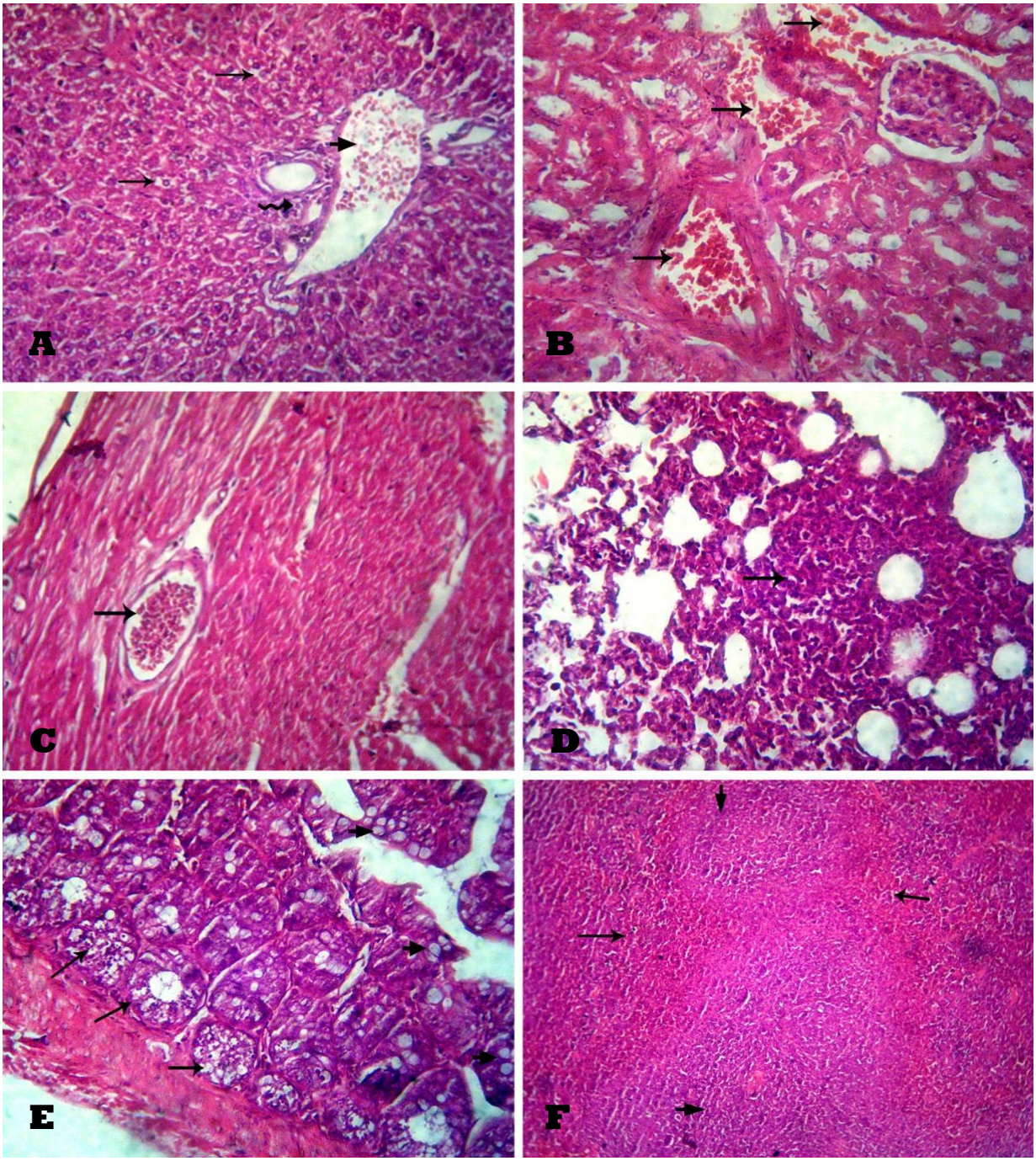

Fig. (3): Chronic toxicity of methanol extract group, one month, shows: liver with mild hydropic degeneration (arrows), slight congestion (arrowhead) and few lymphocytes in the portal area (irregular arrow) (A), kidney with congestion of renal blood vessels (arrows) (B), heart with slight congestion in coronary blood vessel (arrow) with mild perivascular edema (C), lung with slight thickening in the interalveolar septa (arrow) particularly around the alveolar duct (D), intestine with mucinous degeneration in the lining epithelium (arrow heads) and slight activation or metaplasia of Paneth cells in the crypts of Lieberkuhn (arrows) (E) and spleen with normal white pulp (arrow heads) and slightly congested red pulp (arrows) (F). **HE x 400**.

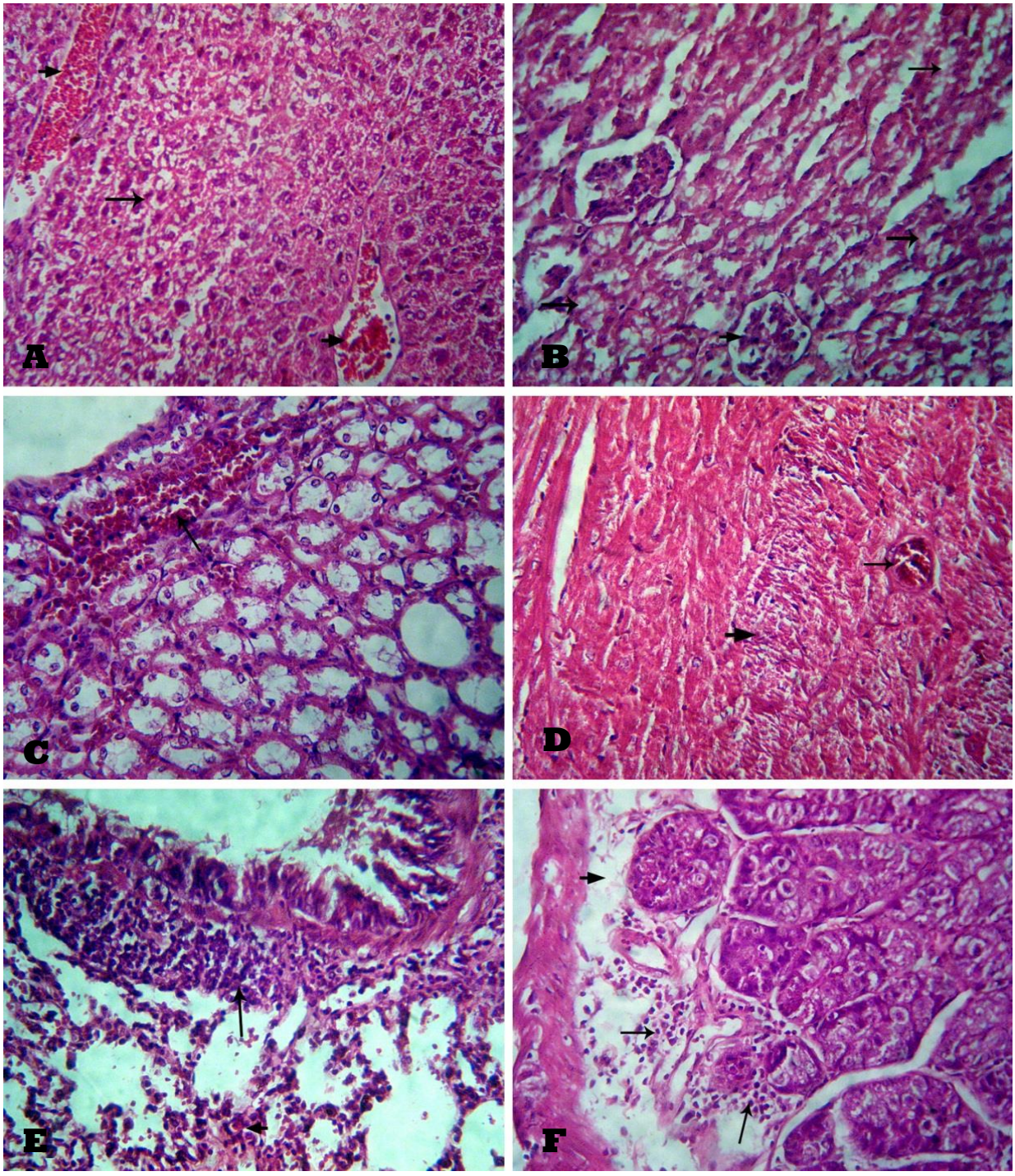

Fig. (4): Chronic toxicity of methanol extract group, 2 months, shows: liver with mild hydropic degeneration (arrow) congestion of hepatic blood vessels (arrowheads) (A), kidney with mild vacuolations and hydropic degeneration in the tubular epithelia (arrows) and normal glomeruli (arrow heads) (B), kidney with hemorrhage beneath the renal pelvis and in the renal medulla (arrow) (C), heart with granular eosinophilic sarcoplasm (arrow head) and congested coronary blood vessel (arrow) (D), lung with peribronchiolar aggregation of round cells (arrow) (E) and intestine (II) with edema (arrow head) and few lymphocytes infiltration in the sub-mucosa (arrows) (F). **HE x 400**.

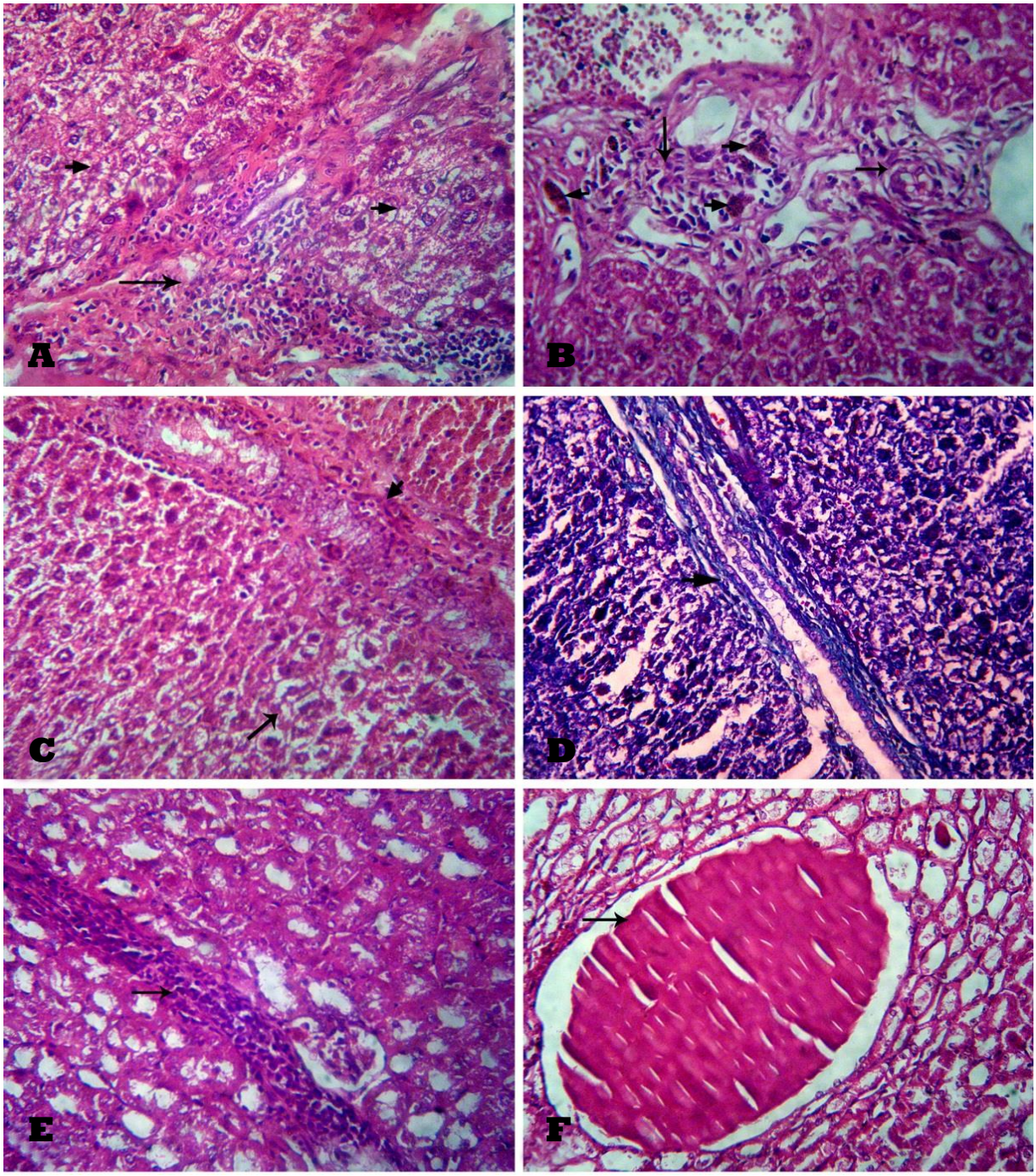

Fig. (5): Chronic toxicity of methanol extract group, three months, shows: liver with moderate hydropic degeneration (arrow heads) and portal round cells infiltrations (arrow) (A), liver with dark brown pigments of hemosiderosis (arrowhead) and fibroblasts proliferation besides few mononuclears (arrows) (B), liver with moderate hyperplasia in the lining epithelium of bile ducts encircled with few fibrous connective tissue (arrow head) and hydropic degeneration (arrow) (C), the fibrous tissue around the hyperplastic bile ducts stained light blue by Masson's trichrome stain (arrow head) (D), kidney with round cells aggregation at corticomedullary junction (arrow) (E) and kidney with cystically dilated renal tubules with eosinophilic material and lined by flattened epithelia (arrow) (F). **HE x 400**.

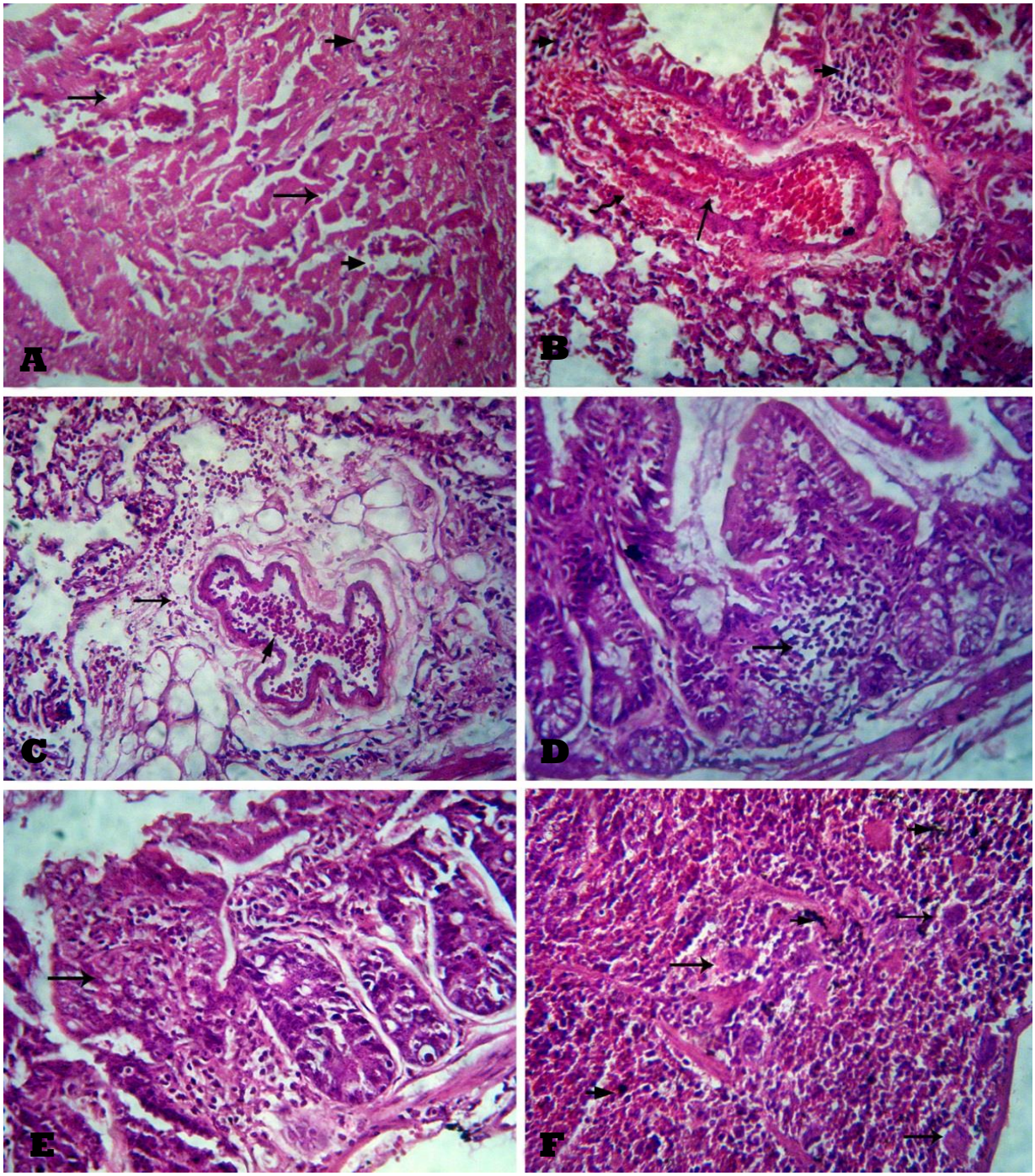

Fig. (6): Chronic toxicity of methanol extract group, three months, shows: heart with irregular areas of Zenker's necrosis (arrows) and hemorrhage (arrow head) (A), lung with peribronchiolar congestion (arrow), hemorrhage (irregular arrow) and lymphocytes infiltrations (arrowheads) (B) besides perivascular edema (arrow) (C), intestine with sub-mucosal round cells infiltration (arrow) (D) and rarely necrosis in the villi (arrow) (E), and spleen with numerous megakaryocytes (arrows) and hemosiderosis of brown pigments (arrow head) (F). **HE x 400**.

CONCLUSION

The findings of this study revealed that there was no toxicity indication of methanol extract of Taif rose during acute, sub-chronic, and chronic periods on physiological parameters, while pathological tests revealed minimal changes in vital organs.From this work, it is obvious that the Taif rose methanol extract could be used as a good natural inexpensive and safe remedy after more *in vitro* and *in vivo* studies.

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Competing interest

Authors have declared that no competing interests exist.

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