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ORIGINAL ARTICLE

Evaluation of Acute Toxicity of Plants' Mixture Used in Traditional Treatment of Kidney Diseases in Morocco

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ABSTRACT

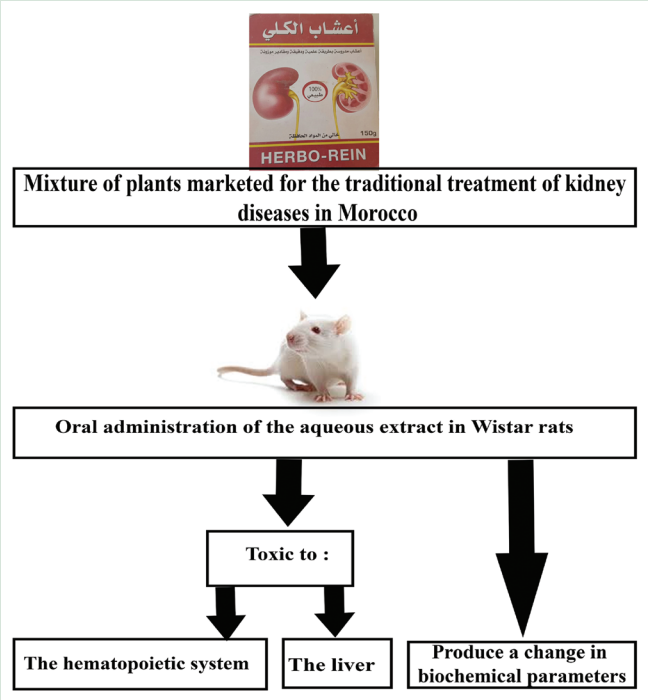
Background: The use of plants' mixture in the traditional treatment of kidney diseases in Morocco is widespread. **Objective:** To evaluate the toxic effects of plants' mixture used in the traditional treatment of kidney diseases in Morocco. **Materials and Methods:** The phytochemical screening was performed. For acute toxicity, single doses of low dose (300 mg/kg), medium dose (500 mg/kg), high dose (2000 mg/kg), lethal dose (6000 mg/kg), and traditional dose (10 ml/kg) body weight of aqueous extracts of plants' mixture were administered orally in Wistar rats. Animals were monitored daily for at least 15 days after an oral administration of aqueous extract of the mixture to detect any changes in body weight, behavior, autonomic profiles, or mortality. Calculation of relative organ weight (ROW), hematological, biochemical analysis, and histopathology evaluation were carried out. **Results:** The acute oral toxicity study showed diarrhea, somnolence, and agitation of different groups of rats, while no mortality and no statistically significant decrease in body weight was observed. Statistically, the kidneys, liver, and spleen showed significant decrease in the ROW of the treated groups of rats when compared to the control group. In biochemical analysis, there was a significant increase in aspartate aminotransferase, creatinine, urea, and uric acid. Hematological parameters showed a significant decrease in leukocytes, eosinophil, basophil, lymphocytes, monocytes, and hematocrit. Histopathological evaluation which revealed major histology changes of liver sections of rats treated with low and medium doses had lymphocyte and plasma cell inflammatory infiltrates; whereas, the liver sections of rats treated with high, lethal, and traditional doses exhibited lymphocyte, plasma cell, and eosinophilic inflammatory infiltrates. **Conclusion:** The study finds that the plants' mixture marketed by herbalists for the treatment of kidney diseases is toxic to body organs such as the liver and the hematopoietic system.

Key words: Acute toxicity, biochemical parameters, hematological analysis, histopathological evaluation, plants' mixture

SUMMARY

- The plants mixture is widely used in the traditional treatment of kidney diseases
- This study aims to evaluate the acute toxicity of the aqueous extract of this mixture of plants
- Effects of aqueous extract on the relative organ weights showed a decrease in relative organ weight of liver, kidney, and spleen
- Effects of aqueous extract on hematological and biochemical parameters proved statistically significant changes in these parameters
- Histopathological evaluation showed the presence of apoptotic bodies and dilated hepatic sinusoids in all histological liver sections of rats treated with the different doses studied whereas histological liver sections of rats treated with doses >2000 mg/kg presented lymphocyte inflammatory infiltrates, plasma cell inflammatory infiltrates, and eosinophilic inflammatory infiltrates
- The results confirmed the toxicity of plants' mixture to the liver the hematopoietic system.

Abbreviations Used: ROW: Relative organ weights, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyltransferase, RBC: Red blood cells, WBC: White blood cells, Hb: Hemoglobin concentration, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, H: Hepatocytes, NS: Normal hepatic sinusoids, CV: Central vein, V: Vein, HP: Hepatic artery, BD: Bile ducts, AB: Apoptotic bodies, DS: Dilated hepatic sinusoids, LI: Lymphocytic infiltrate, PI: Plasmacytic infiltrate, EI: Eosinophilic infiltrate, US: Urinary space, G: Glomerulus, DCT: Distal convoluted tubule, PCT: Proximal convoluted tubule.



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INTRODUCTION

Practice of phytotherapy is currently in full swing in Morocco. They are experiencing a growing interest of various origins, related to multiple issues. On the one hand, Morocco has high plant species richness with about 42,000 including nearly 600 used in traditional medicine;[1] on the other hand, illiteracy, the limited income of the Moroccan population, and in general, sociocultural factors have increased the demand for treatment by plants.[2] Thus, modern medicine, sometimes inaccessible for its cost or unable to treat a serious or chronic disease, gives way to the practice of herbalists and traditional healers. Unfortunately, poor diagnosis of the diseases and the use of herbal medicine lead to serious risks or even fatal poisoning. This is explained by the phytotherapy of several diseases, including diabetes,[3,4] hypertension,[3,4] cardiovascular diseases,[5] and kidney diseases.[6]

Studies on herbal medicine in the region of Fez-Meknes are very rare, and studies on toxicological aspects are rare or absent, with the exception of an ethnobotanical survey conducted in the city of Fez in central Morocco by a single herbalist who revealed that the majority of medicinal plants were used against urinary disorders (21%), followed by diseases of the digestive system (19.6%) and rheumatological diseases (18.2%).[7]

While research in phytotherapy of kidney diseases in Morocco is limited, this disease is common. It affects almost 2.9% of the Moroccan adult population, according to the Maremar (Maladies rénales chroniques au Maroc).[8]

We conducted an ethnopharmacological study on the phytotherapy of renal diseases in Fez-Meknes region to deepen and enrich the knowledge about the plants used. We found that the mixtures of plants are packaged and labeled highly recommended by herbalists [Figure 1]. The method of preparation is the infusion of four spoons of the mixture into a liter of water and drinking it three times a day.

The aim of the present study is to identify the chemical compounds and experimentally evaluate the toxic effect of aqueous extract of the mixture used in the phytotherapy of renal diseases in Wistar rats.

MATERIALS AND METHODS

Plants' mixture materials

The mixture of plants was purchased from herbalists in the Fez-Meknes region and crushed [Figure 1].

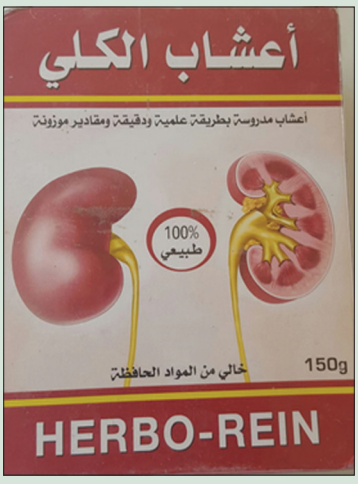

Figure 1: Plants' mixture highly recommended and marketed by herbalists to treat kidney diseases in Fez-Meknes region

Preparation of aqueous extracts

The plants' mixture was powdered, and extraction was performed by infusion. Indeed, 10 g of the mixture extract was added to 100 ml of water during 30 min of boiling and then filtered and concentrated using a Rotavapor.

Phytochemicals' screening

The extract is screened for phytochemical constituents (coumarins, leucoanthocyans, flavonoids, mucilags, tannins, sterols and terpenes, quinones, and cardiac glycosides) using a simple qualitative method as described by Diallo[9] and Paris and Nothis.[10] The extract is concentrated, and it was dried under low pressure. Then, the appearance color of the extracts was noted.

Acute toxicity

Acute toxicity assessment was performed on 60 Wistar rats through the oral route, divided into 6 groups with 10 rats in the group (5 males and 5 females) weighing between 60 and 120 g.

Group 1 of 10 rats (5 males and 5 females) served as the control group receiving normal saline; for Groups 2, 3, and 4, each group of 10 rats (5 males and 5 females) received, respectively, a single oral dose of 300 (low dose), 500 (medium dose), and 2000 mg/kg (high dose) body weight of aqueous extract of plants' mixture.

The fifth group of 10 rats (5 males and 5 females) received a single oral dose of 10 ml/kg (traditional dose) (According to the traditional use of the mixture [dose of 200 ml for an adult person of 60 kg, three times a day]).

The sixth group of 10 rats (5 males and 5 females) received a single oral dose of 6000 mg/kg (lethal dose) body weight of aqueous extract of plants' mixture (the dose chose theoretically for lethal dose [LD_{50}] by multiplying the high dose [2000 mg/kg] three times).

The animals were observed for any behavioral changes, neurological, autonomic profiles, and mortality. Body weight was measured daily during the 15 days following the aqueous extract of plants' mixture administration.

Calculation of relative organ weights

The relative organ weights (ROWs) in comparison with the control group was calculated by the following formula:

ROW = (organ weight/body weight) × 1000.[11]

Hematological and biochemical analysis

The rats were anesthetized, and a blood sample was taken by cardiac puncture. Blood was collected in tubes containing ethylenediaminetetraacetic acid for hematological analysis and nonheparinized tubes for biochemical analysis. The biochemical parameters measured were alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, total bilirubin, direct bilirubin, gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatinine, urea, uric acid, uric acid, high-density lipoprotein cholesterol (HDL cholesterol), cholesterol total, levels of magnesium, ferritin, phosphorus, and serum iron. Hematologic parameters measured were red blood cells (RBC), white blood cells, hemoglobin concentration (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelets, neutrophils, lymphocytes, eosinophils, and monocytes.

Histopathology evaluation

The animals are anesthetized by "intraperitoneal" with sodium pentobarbital at the dose of 30 mg/kg, before making their decapitation

using guillotines which must be sharpened and adjusted frequently to ensure proper performance.

The organs were excised and weighed. The relative weight of the organ has been calculated. Vital organs such as kidneys and liver were preserved in 10% neutral formol for histopathological reasons.

Statistical analysis

Data were expressed as mean values ± standard error of the mean. Statistical significance was determined with the *t*-test of Student. $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening of aqueous extract of mixture revealed the presence of tannins, catechic tannins, gallic tannins, saponins, flavonoids, leucoanthocyans, catechols, coumarins, alkaloids, cardiac glycosides, and mucilage. However, free quinones were not detected [Table 1].

Acute toxicity

Effects of clinical signs of toxicity

The acute oral toxicity study showed diarrhea after 2 h and somnolence after 3 h of acute administration of high, lethal, and traditional doses; the rats were agitated after 24 h for low, medium, high, lethal, and traditional dose. When, no deaths, no constipation, no edema, no tremor, no convulsion were observed [Table 2].

Table 1: Phytochemical screening of aqueous extract of mixture

Chemical compounds	Result
Total tannins	+
Catechic tannins	+
Gallic tannins	+
Saponins	+
Flavonoïds	+
Leucoanthocyans	+
Catechols	+
Coumarins	+
Alcaloïds	+
Cardiac glycosides	+
Free quinones	-
Mucilages	+

+: Present; -: Absent

Effects of body weight

No statistically significant decrease in body weight was observed during the 14 days of acute administration for low, medium, high, lethal, and traditional dose [Table 3].

Effects of aqueous extract of mixture on relative organ weights

After 14 days, the rats were sacrificed, and their organs (liver, kidney, and spleen) were immediately removed for weight organ examination.

Statistically, kidney, liver, and spleen showed a significant decrease in the ROW of the treated groups of rats compared to the control group [Figure 2].

Effects of aqueous extract of mixture on biochemical parameters

There was a significant increase in AST, creatinine, urea, uric acid in the animal groups were received the different doses compared to the control group. Whereas, ALT, ALP, triglycerides, magnesium, and phosphorus were decreased significantly. In addition, the ferritin and serum iron were increased in traditional dose only.

There was no significant change in total protein, total bilirubin, direct bilirubin, GGT, total cholesterol, and HDL-cholesterol [Table 4].

Effects of aqueous extract of mixture on hematological parameters

Figure 3 showed a significant decrease in leukocytes, eosinophil, basophil, lymphocytes, monocytes, and hematocrit in all groups of rats receiving the different doses administered. Whereas, neutrophil and platelets were significantly increased when compared with normal rats. Erythrocytes (RBC) count and hemoglobin (HGB) level and MCHC were significantly increased in high dose, lethal dose, and traditional dose. However, the MCV was significantly increased in medium, high, lethal, and traditional dose.

Effects of aqueous extract of mixture on histology of liver

Figure 4a showed the normal histology of the central vein and hepatic lobule formed by hepatocyte cords separated by hepatic sinusoids. Figure 4b showed the normal histology of the portal region of the liver with the bile ducts (BD), vein, and hepatic artery (HP).

All the groups of rats treated with the different doses studied have in their liver's apoptotic bodies (AB) [Figure 4c] and dilated hepatic

Table 2: Results of animal observation during the first 24 h and every day for 14 days after oral administration of the aqueous extract of mixture

	Clinical signs of toxicity							
	Diarrhea (h)	Constipation	Agitation (h)	Edema	Tremor	Convulsion	Somnolence (h)	Death
Control	-	-	-	-	-	-	-	-
Low dose	-		After 24	-	-	-	-	-
Medium dose	-	-	After 24	-	-	-	-	-
High dose	After 2	-	After 24	-	-	-	After 3	-
Lethal dose	After 2	-	After 24	-	-	-	After 3	-
Traditional dose	After 2	-	After 24	-	-	-	After 3	-

-: Absent

Table 3: Effects of acute administration on body weight after 14 days of treatment with extract aqueous of mixture

	Lethal dose	Traditional dose	Low dose	Medium dose	High dose
Day 1	161.6±16.9	159.±10.8	162.4±7.2	155±9.7	171±7.6
Day 7	151.4±13.7	158±8.9	159.6±6.7	154.2±12.2	165±8.9
Day 10	151.6±14	143±8.4	158.6±7.2	151.6±11.8	164.4±10.1
Day 14	146.4±13.1	139.6±9.5	154±10.1	150.8±10.2	158.2±10.8
Significant	NS	NS	NS	NS	NS

NS: Not significant

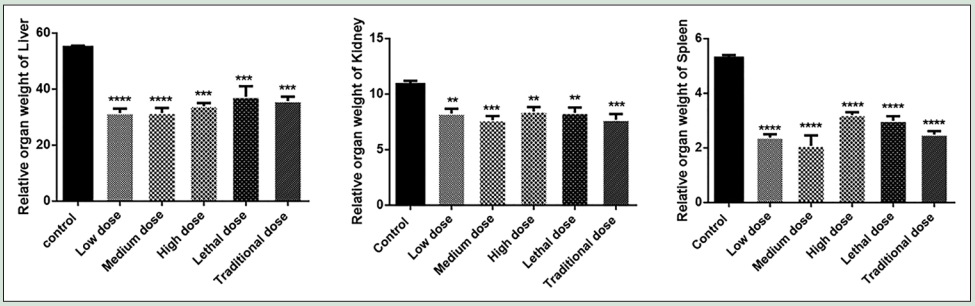

Figure 2: Relative organ weights of kidney, liver, and spleen of rats after 14 days of treatment with aqueous extract of the mixture. Value was expressed as the mean ± standard error of the mean. Comparisons of means were performed using the test of Student. ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, *n* = 3 for each group

Table 4: Effect of aqueous extract of mixture on biochemical parameters

	Control	Low dose	Medium dose	High dose	Lethal dose	Traditional dose
Liver profile						
ALT (U/l)	61.00±5.00	44.00±2.10*	45.00±2.00*	44.00±2.62*	42.00±3.70*	43.50±1.35*
AST (U/l)	211.50±2.50	243.00±8.00*	265.50±16.50*	417.50±17.50***	311.00±11.00***	315.00±7.00***
Total Protein (g/l)	45.50±2.4	46.50±2.50^{NS}	52±1.00^{NS}	50.50±1.50^{NS}	51.50±1.50^{NS}	48.50±1.50^{NS}
Total Bilirubin (mg/l)	1.00±00.00	1.00±00.00^{NS}	1.00±00.00^{NS}	1.00±00.00^{NS}	1.00±00.00^{NS}	1.00±00.00^{NS}
Direct Bilirubin (mg/l)	1.00±00.00	1.50±0.50^{NS}	1.00±00.00^{NS}	1.00±00.00^{NS}	1.00±0.00^{NS}	2.00±0.00^{NS}
Alkaline phosphatase (U/l)	412.00±11.00	237.00±13.50***	257.00±8.00***	274.00±19.00**	268.00±3.00***	225.00±10.00***
GGT (U/l)	4.00±00.00	4.00±00.00^{NS}	4.00±00.00^{NS}	4.00±00.00^{NS}	4.00±00.00^{NS}	4.00±00.00^{NS}
Renal profile						
Creatinine (mg/l)	3.66±0.33	4.98±0.08*	5.68±0.11**	5.10±0.17*	5.72±0.10**	5.66±0.33*
Urea (g/l)	0.33±0.01	0.38±0.01*	0.44±0.03*	0.61±0.02***	0.42±0.02*	0.42±0.02*
Uric acid (mg/l)	13.50±1.00	19.50±1.00*	20.00±1.52*	21.00±1.5*	26.00±2.05**	21.00±1.00**
Lipids profile						
Total cholesterol (g/l)	0.59±0.05	0.47±0.005^{NS}	0.48±0.04^{NS}	0.50±0.02^{NS}	0.54±0.01^{NS}	0.55±0.04^{NS}
HDL-cholesterol (g/l)	0.21±0.02	0.17±0.01^{NS}	0.21±0.01^{NS}	0.19±0.01^{NS}	0.22±00.01^{NS}	0.22±0.005^{NS}
Triglycerides (g/l)	0.73±0.07	0.27±0.01**	0.19±0.01**	0.24±0.01**	0.16±0.01**	0.26±0.01**
Electrolytes						
Magnesium (mg/l)	33.50±2.50	20.22±0.64**	21.29±0.75**	20.42±0.75**	20.10±0.02**	21.10±1.31*
Phosphorus (mg/l)	140.00±7.00	40.00±3.00***	53.33±3.33***	58.00±3.00***	48.00±2.00***	40.00±3.00***
Ferritin (ng/l)	15.00±0.57	12.75±1.15^{NS}	10.70±1.00^{NS}	16.00±1.30^{NS}	16.05±0 .65^{NS}	26.25±2.45*
Serum iron (µg/l)	1.64±0.10	1.85±0.13^{NS}	1.77±0.15^{NS}	1.50±0.10^{NS}	1.96±0.12^{NS}	2.42±0.14*

Values are expressed as mean±SEM, *n*=3. **P*<0.05; ***P*<0.01; ****P*<0.001. AST: Aspartate transaminase; ALT: Alanine aminotransferase; GGT: Gamma glutamyltransferase; NS: Not significant; SEM: Standard error of mean; HDL: High-density lipoprotein

sinusoids [Figure 4d]. In addition, the liver sections of rats treated with low and medium doses had lymphocyte and plasma cell inflammatory infiltrates [Figure 4e], whereas the liver sections of rats treated with high, lethal, and traditional doses exhibited lymphocyte inflammatory infiltrates, plasma cell inflammatory infiltrates, and eosinophilic inflammatory infiltrates [Figure 4f]. Furthermore, no structural change was observed for the BD, vein, and HP in the portal area and no fatty liver, fibrosis, biliary sludge, and hemosiderin were present in all the treated group.

Effects of aqueous extract of mixture on histology of kidney

Figure 5a showed the normal histology of the kidney with the normal cortex, distal convoluted tubule, proximal convoluted tubule, and glomerulus. No structural change was observed in all groups treated at all dose [Figure 5b].

DISCUSSION

Phytochemical screening of the aqueous extract of the mixture revealed the presence of all desired chemical compounds except free quinones. The presence of these compounds can cause serious damage to vital organs such as the liver and kidney.

Several studies have shown that the daily use of megadoses of flavonoids and isoflavonoids causes cytotoxicities and mitochondrial toxicity due to phenolic cycles that give phenoxyl radicals.[12] Other studies have shown hepatotoxicity in mice caused by flavonoid epigallocatechin gallate and phenolic propyl gallate.[13]

The results of the acute oral toxicity study showed diarrhea after 2 h and somnolence after 3 h of acute administration of high, lethal, and traditional doses; the rats were agitated after 24 h for the low, medium, high, lethal, and traditional dose. The presence of signs such as diarrhea and agitation can be explained by sensitivity to the substances, where risk of toxicity is present. Concerning the body weight, the decrease in body weight can be explained by a decrease in the weight of internal organs due to exposure to toxic substances.[14,15] Statistically, our study finds that there is no significant decrease in the body weight of all groups of rats.

Changes in the weight of internal organs were considered as an indicator of chemical exposure;[16] during our study, a statistically significant decrease was noted for the liver, kidneys, and spleen, suggesting a risk of acute toxicity.

To confirm the risk of toxicity, several other analyses were performed, including biochemical and hematological tests. In the biochemical

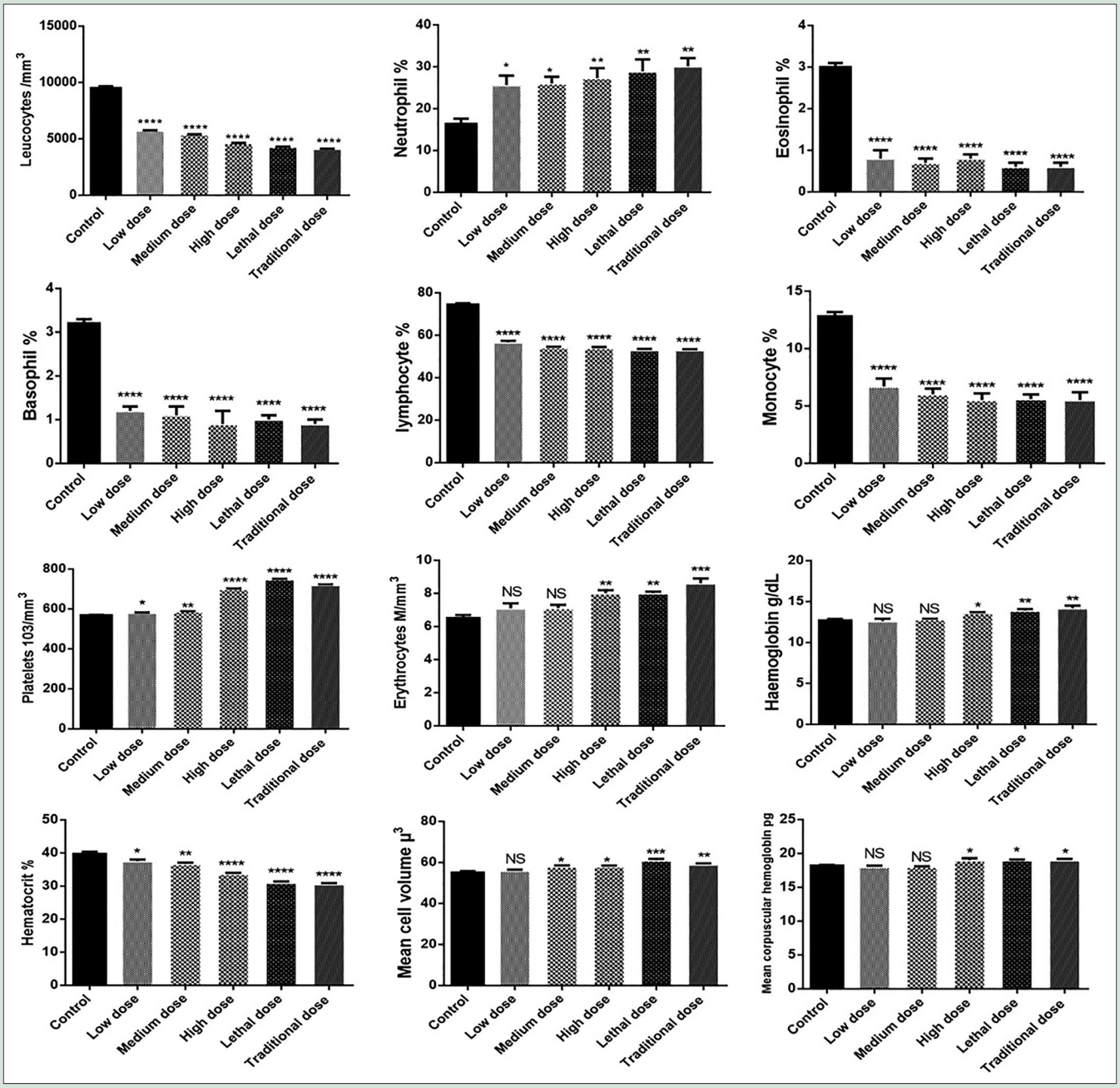

Figure 3: Hematological parameters of rats after 14 days of treatment with aqueous extract of the mixture. Values are expressed as mean ± standard error of the mean, $n = 3$; NS: Not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

analyzes, the hepatic damage was examined by the level of transaminase enzymes.[17]

Furthermore, acute administration of the aqueous extract of mixture significantly increased the level of ALT and AST in the serum of all animals in the treatment group compared to animals in the control group. ALP is another liver enzyme; an increase in their level is an indication of cellular damage and loss of functional integrity of hepatocyte cell membranes;[18] in our study, the ALP level was significantly increased in the serum of animals from all treated groups.

In the renal profile, the levels of creatinine and urea in the plasma are key indicators of renal abnormality. In this study, a statistically significant increase in creatinine and urea in all groups of rats when compared with the control group. Uric acid is another indicator of kidney function. However, acute oral administration of aqueous extract of mixture significantly increases the uric acid level as compared to the control group.

In the lipid profile, several studies have been linked between changes in lipid levels in the blood and renal damage[19,20] when another study has linked high triglyceride levels with the development of fatty liver disease.[21] In our study, the level of total cholesterol and HDL-Cholesterol did not change, while the triglyceride level was significantly decreased in all groups of rats when compared to the control group. Moreover, in the electrolytes, several renal diseases are associated with decreased electrolytes such as magnesium.[22] In this study, the level of electrolytes (magnesium and phosphorus) increased significantly in

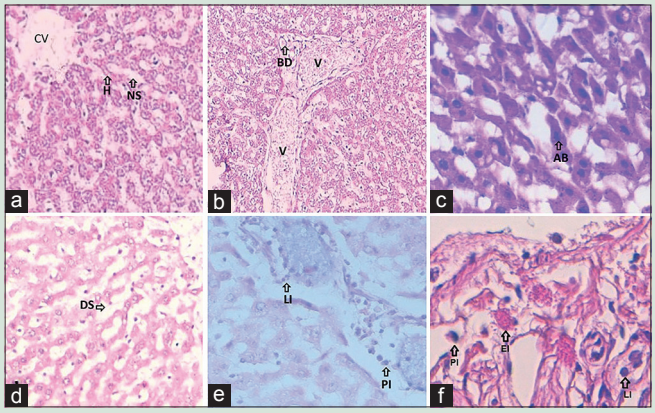

Figure 4: Photomicrographs of liver sections of control rats ([a] hepatic lobule stained with HES, ×10, [b] portal area stained with HES, ×20), (c) photomicrographs of liver sections of group treated at 2000 mg/kg body weight (stained with HES, ×40), (d) photomicrographs of liver sections of group treated at 500 mg/kg body weight (stained with HES, ×20), (e) Photomicrographs of liver sections of group treated at 300 mg/kg body weight (stained with HES, ×40), (f) photomicrographs of liver sections of group treated at lethal dose (stained with HES, ×40). H: Hepatocytes, NS: Normal hepatic sinusoids, CV: Central vein, V: Vein, HP: Hepatic artery, BD: Bile ducts, AB: Apoptotic bodies, DS: Dilated hepatic sinusoids, LI: Lymphocytic infiltrate, PI: Plasmacytic infiltrate

all five rat groups receiving extract of the mixture, while the ferritin and serum iron levels increased only in the group of rats receiving the lethal and the traditional dose.

The analysis of hematological parameters is necessary to evaluate the risk of alteration of the hematopoietic system by a toxic compound.[23] Oral administration of the aqueous extract of the mixture leads to a stasis of hematopoiesis which is explained by a significant increase in the number of RBC in the groups of rats receiving the high, lethal, and traditional dose and a significant increase in the platelets in all rat groups when compared with control group. There was a significant decrease of leukocytes, eosinophils, basophils, lymphocytes, and monocytes, which is explained by a weakness of the immune system. On the other hand, the increase in neutrophils can be explained by the body's reaction against inflammation.

Death of hepatocytes occurs mainly by apoptosis or necrosis. Necrosis is characterized by an increase in cell volume organoleptic swelling and early rupture of the plasma membrane with loss of intracellular content.[24] Cell death ends with the formation of organs containing organelles, named AB.

Sinusoids are highly specialized capillaries that provide vascularization of the hepatic lobule when hepatic dilatation is characterized by enlargement of the capillaries at the hepatic lobules. During our studies, the administration of the aqueous extract of the mixture causes dilation of the liver sinusoids in all groups of rats which received the different doses when compared to the control group.

Inflammation involves cells, vessels, and changes in the extracellular matrix, and many pro-inflammatory or anti-inflammatory chemical mediators can alter or maintain the inflammatory response. However, the inflammatory reaction involves certain immune cells such as lymphocytes, plasma cells, monocytes and macrophages, eosinophilic granulocytes, basophils, and mast cells.

A remarkable inflammatory infiltrate of lymphocyte and plasma cell types was found in the hepatic sections of the low dose group when the lethal dose groups represent inflammatory infiltrates of lymphocytes, plasma cells, and eosinophils.

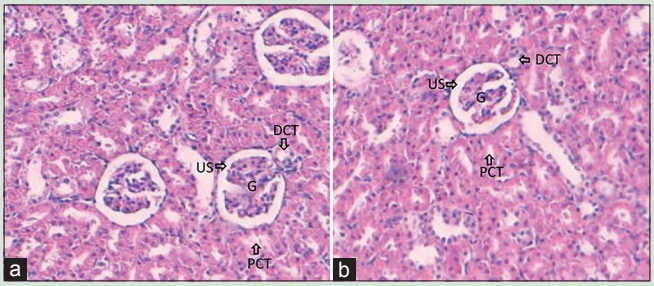

Figure 5: (a) Photomicrographs of kidney sections of control rats (stained with HES, ×10), (b) Photomicrographs of kidney sections of group treated at 6000 mg/kg body weight (stained with HES, ×10); US: Urinary space, G: Glomerulus, DCT: Distal convoluted tubule, PCT: Proximal convoluted tubule

CONCLUSION

The present study has confirmed the toxicity of this plants' mixture marketed by herbalists for the traditional treatment of kidney disease. For kidney tests, our research has confirmed a change in biochemical parameters such as creatinine, urea, and uric acid, but no histologic changes were observed. On the other hand, liver tests confirmed a change in some biochemical parameters and histologic changes in the liver. Hence, the need for the sensitivity of herbalists and consumers the danger of this plants mixture.

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Note

The opinions expressed in the article are those of the authors and contributors, and do not necessarily reflect those of the journal owner, the editors, the editorial board, or Publisher.

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Conflicts of interest

There are no conflicts of interest.

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