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Assessment of the Effects of Khat Extract on the Hypouricemic Activities of Allopurinol in Rats

Safia Abdulatef A. Alrezami[1,3] *, Abdulmalik Abdullah Y.Abudunya[1], Hassan Mohammed Al- Mahbashi[2] and Abdulatef Mohammed M. Abbas[1,3]

[1]**Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Science, Sana'a University, Sana'a, Yemen,**
[2]**Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine and Health Science, Sana'a University, Sana'a, Yemen,**
[3]**Department of Pharmacy, Faculty of Medicine and Health Science, Al-Mahweet University, Al-Mahweet, Yemen.**

*Corresponding author: *Safiaabdulatef@gmail.com*

Abstract

Background: Hyperuricemia is an important metabolic condition that is closely associated with gout and cardiovascular disorders. The first-line treatment is still allopurinol, a xanthine oxidase inhibitor, but little is known about how it interacts with herbal substances. Khat (*Catha edulis*), which is traditionally consumed in East Africa and the Arabian Peninsula, has recently spread throughout the world because of migration. The purpose of this study was to assess how khat extract affects the hypouricemic action of allopurinol in hyperuricemia rats.
Methods: Forty-eight male albino rats were randomly divided into six groups and given different amounts of allopurinol, khat extract, yeast extract and potassium oxonate. Biochemical investigations included serum and urine analyses for uric acid levels, xanthine oxidase activity, kidney and liver function tests, urine profile, and complete blood count. Histopathological examinations of renal and hepatic tissues were also conducted.
Results: Khat extract significantly elevated the serum uric acid level and xanthine oxidase activity. Moreover, its combination with allopurinol reduced the drug's hypouricemic efficacy. Coadministration also induced more pronounced alterations in renal and hepatic function parameters, together with histopathological evidence of tissue injury.
Conclusion: Khat extract reduced the therapeutic effectiveness of allopurinol and aggravated both biochemical abnormalities and histological damage under hyperuricemia conditions. These outcomes, based on an animal model, point to a possible interaction between khat and allopurinol and emphasize the need for further investigation in humans.

Article Info

Keywords:
Catha edulis, Allopurinol, Interactions, Hypouricemic activity, biochemical and histology alterations, Rat model.

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1. INTRODUCTION

Allopurinol is the primary treatment for hyperuricemia [1]. It has been extensively used in clinical practice for over 50 years, mainly for the management of gout and tumor lysis syndrome [2]. It has also been the cornerstone of uric acid–lowering therapy for more than three decades [3]. It is commercially available as an anti-gout drug. Allopurinol works by inhibiting xanthine oxidase and is indicated when uricosuric agents fail to lower serum urate levels below 7.0 mg/dL [4]. Traditionally, it is administered in two or three doses totaling 300 mg/day, although recent practice often favors a single daily dose of 300 mg [5]. Severe adverse effects are uncommon but are of concern because of their high incidence, including skin rashes, elevated eosinophil and white blood cell counts, fever, liver inflammation, and kidney dysfunction [6]. In addition to allergic reactions, allopurinol may induce liver necrosis, renal impairment, gastrointestinal issues, and necrosis of the skin and mucous membranes [1]. This drug is known to interact with several medications, including azathioprine, mercaptopurine, ACE inhibitors, thiazide diuretics, penicillin antibiotics, and warfarin [7]. However,

research on the interactions between allopurinol and herbal products remains limited.

One widely used plant is khat (*Catha edulis*), which is cultivated in Eastern Africa and the Southwest Arabian Peninsula, and whose global use has expanded due to migration [8]. Khat leaves are consumed for their amphetamine-like stimulant effects [9], and it is estimated that 10–20 million people worldwide use khat, with its use spreading to countries such as Australia and the USA [10]. Despite allopurinol's established role as a standard therapy, little is known about its interaction with herbal substances such as khat. Therefore, this study aimed to investigate the effect of khat extract on the hypouricemic activity of allopurinol in rats

2. MATERIALS AND METHODS

2.1. Sample collection and extract preparation

A total of 1.5 kg of fresh, pesticide-free *Catha edulis* shoots were collected from Wadi Aljannet, Ibb, Yemen. The plant material was identified by Dr. Hassan M. Ibrahim, a taxonomist, who also provided a voucher specimen (740a) that was deposited in the Laboratory of Botany at Sana'a University, Yemen. The shoots were then rinsed with distilled water to remove dust and impurities.

The buds were removed from the fresh khat leaves, which were then cleaned and pulverized using a blender. A rotary shaker was used to hold the crushed material for 20 h after it was soaked in 99.6% methanol (Techno Pharmchem, India). The filtering process was performed in two stages: first, large particles were removed with gauze, and then premium filter paper was used. The remaining plant material was re-extracted using fresh methanol, and the resulting filtrate was combined. Methanol was then removed from the extract using a rotary vacuum evaporator at 65℃. The semisolid extract was then freeze-dried and stored as a powder. Before use, the powder was dissolved in distilled water to create a new solution [11].

2.2. Phytochemical Tests of Khat (*Catha edulis*)

Phytochemical screening of *Catha edulis* extract was performed to identify the major bioactive constituents using standard qualitative methods described by Shaikh and Patil (2020). The extract was tested for alkaloids, tannins, flavonoids, phenolic compounds, terpenoids, and glycosides using established colorimetric assays. All assays were conducted using appropriate aqueous or alcoholic solutions of the extract, following previously reported protocols [12].

2.3. Animal experiments

2.3.1. Housing of animals

A total of 48 healthy 4-week-old male albino rats weighing 140–160 g were obtained from the Animal Laboratory of the Faculty of Medicine, Sana'a University, Sana' a, Yemen. The rats were housed in a facility and acclimated for two weeks at a temperature of 20 ± 4℃ and a 12-h light/12-h dark cycle. The rats had unlimited access to tap water and rodent food ad libitum. The rats were housed according to the guidelines for proper conduct of animal experiments.

2.3.2. Induction of hyperuricemia

Hyperuricemia was induced by administering yeast extract (10 g/kg) orally twice daily, along with intraperitoneal injections of potassium oxonate (PO) (250 mg/kg) on days 1, 3, and 7 for one week [13, 14].

2.3.3. Grouping and dosing

The rats were randomly divided into six groups (n=8 each) [15]:

Group I (normal control [NC] group) received 1 ml of distilled water for 28 days. Group II: Khat extract (500 mg/kg [16]) was administered orally for 28 days. Group III hyperuricemia control (PC) was induced with yeast extract (10 g/kg, orally administered twice daily for 7 days [14]) and potassium oxonate (1 ml, i.p.) on days 1, 3, and 7 [13]. Group IV: Hyperuricemia + khat extract: After induction, 500 mg/kg khat extract was administered orally for 28 days [16]. Group V allopurinol (HUA) group: After induction, 5 mg/kg [17, 18] allopurinol was administered orally for 28 days. Group VI received khat extract + allopurinol (HUA); after induction, 500 mg/kg khat extract [16] and 5 mg/kg allopurinol [17, 18] were administered orally for 28 days [6].

2.3.4. Sample and blood collection

At the end of the study period (on the 29th day), urine samples were collected for 2-hour period in a metabolic cage. Subsequently, the animals were anesthetized via intraperitoneal injection of xylazine-ketamine (XK) (5 mg/kg i.p; 50 mg/kg i.p) [19]. Blood samples (3 mL) were collected through cardiac puncture. Blood was placed in both EDTA tubes (for hematology) and plain tubes (for serum separation), and the samples were centrifuged and stored at -20℃ until analysis. The rubber tube without an anticoagulant was kept at room temperature for 20 min and centrifuged at 6000 rpm for 5 min to obtain the serum.

2.3.5. Biochemical analysis:

Uric acid was measured using commercial kits (Spinreact S.A.U., REF: 41000, Lot: LIQ775), whereas xanthine oxidase (XO) activity was determined using Solarbio Life Sciences kits (Cod: BC1095, China). Renal function

tests included serum creatinine (BioSystems, Spain), blood urea nitrogen (BUN; BioSystems, Spain), urine analysis, total protein, and albumin (BioSystems, Spain). Liver function tests included AST, ALT, ALP, and total bilirubin. Complete blood count (CBC) parameters, including red blood cell (RBC) count, hemoglobin (Hb) level, white blood cell (WBC) count, differential leukocyte count, and platelet count, were also assessed.

2.3.6. Histopathology evaluation

After blood samples were collected, all rats were sacrificed humanely to assess gross and histopathological alterations in the kidney and liver. Tissue samples were collected and preserved in 10% formalin for histopathological examination [17]. Tissues were prepared for routine hematoxylin-eosin (H&E) and periodic acid–Schiff (PAS) staining. The tissue was evaluated by a histopathologist who was blinded to the treatment groups.

2.3.7. Ethical Consideration

All procedures followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH). The study was approved by the Ethical Committee of the Faculty of Medicine and Health Sciences, University of Sana'a (Approval Number-433-Date:15-02-2025).

2.3.8. Statistical analysis

The results are expressed as means ± standard deviations (SDs), and comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests. Urine parameters were analyzed using the chi-square test. The difference between groups was set at $P \leq 0.05$ using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Phytochemical Screening Results

The *Catha edulis* plant contains different constituents that appeare in Table (1).

Table[1]: Results of phytochemical tests for *Catha edulis*.

Types	*Catha edulis*
Alkaloids	+
Tannins	+
Flavonoid	+
Terpenoids	+
Phenolic compounds	+
Glycosides	+

+ present

3.2. Induction of Hyperuricemia

As shown in Figure (1), all hyperuricemia-induced groups exhibited significantly higher serum uric acid levels than the control and khat extract groups.

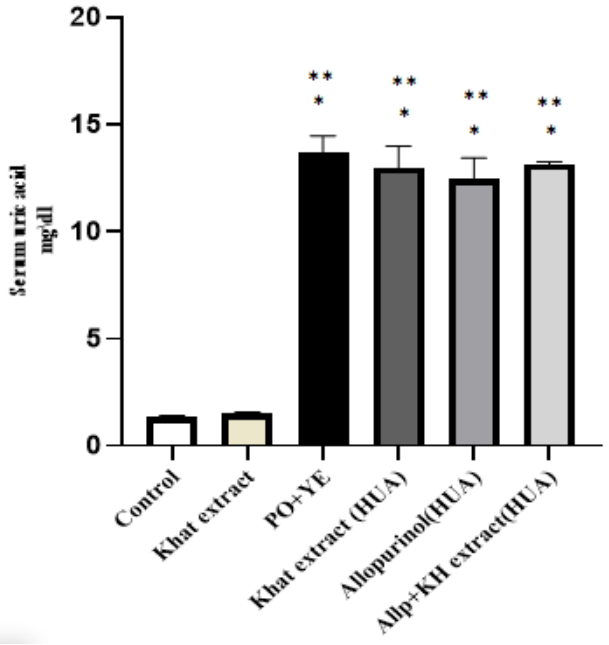

Figure 1. Serum uric acid levels after induction of hyperuricemia.

Data are expressed as the mean ± standard deviation (n = 8 per group).
PO+YE: potassium oxonate + yeast extract. Khat extract (HUA): khat extract with hyperuricemia.
Allp +Khat extract(HUA): allopurinol + Khat extract with hyperuricemia.
* $p < 0.001$ vs. control.
** $p < 0.001$ vs. Khat extract.

3.3. Effect of Khat Extract and Allopurinol on Serum Uric Acid Levels

As shown in Figure (2), serum uric acid levels were significantly higher in the PO+YE ($p = 0.001$ vs. control) and khat extract–HUA groups ($p = 0.001$ vs. control and vs. khat extract) than in the control and khat extract groups. The allopurinol plus khat extract group showed lower levels than those in the two groups but remained higher than those in the control ($p = 0.018$ vs. allopurinol plus khat extract). Comparisons between PO+YE and allopurinol, as well as khat extract–HUA and allopurinol, were also significant ($p = 0.001$).
PO+YE: potassium oxonate plus yeast extract. Khat extract (HUA): khat extract under hyperuricemia conditions.
Allp+Khat extract (HUA): allopurinol with khat extract under hyperuricemia conditions.
* $p = 0.05$ vs. Control

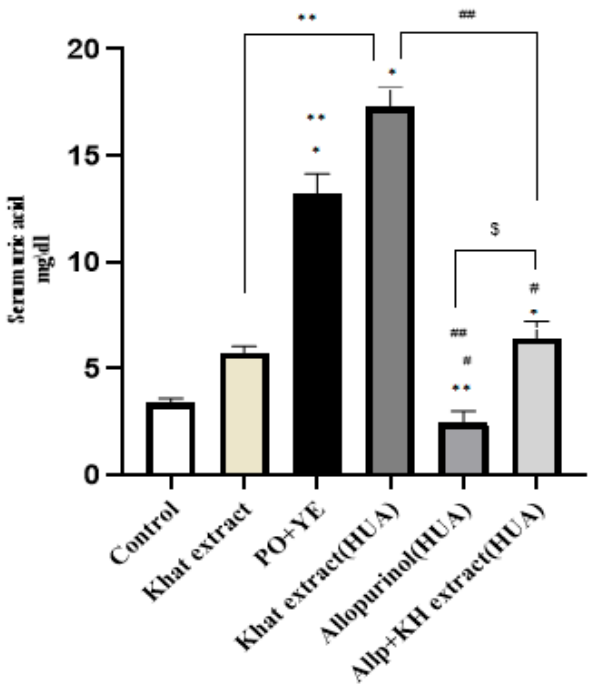

Figure 2. Serum uric acid levels (mg/dl) in different experimental groups (n = 8 per group).

** p = 0.05 vs. Khat extract

p = 0.05 vs. PO+YE

p = 0.05 vs. Khat extract-HUA

$ p = 0.05 vs. Allopurinol + Khat extract)HUA)

3.4. Effect of Khat Extract and Allopurinol on Urine Uric Acid Levels

As shown in Figure (3), urinary UA levels were significantly higher in the PO+YE and khat extract–HUA groups than in the control and khat extract groups ($p \leq 0.03$ vs. control; $p \leq 0.0006$ vs. khat extract). The khat extract–HUA group showed the highest levels, which were significantly greater than those in the PO+YE, allopurinol, and allopurinol plus khat extract groups ($p < 0.0001$). The allopurinol and allopurinol plus khat extract groups exhibited lower values.

PO+YE: potassium oxonate plus yeast extract.

Khat extract(HUA): khat extract under hyperuricemia conditions.

Allp+Khat extract (HUA): allopurinol with khat extract under hyperuricemia conditions

* p = 0.05 vs. Control,

** p = 0.05 vs. Khat extract,

p = 0.05 vs. PO+YE,

p = 0.05 vs. Khat extract-HUA,

$ p = 0.05 vs. Allopurinol + Khat extract(HUA)

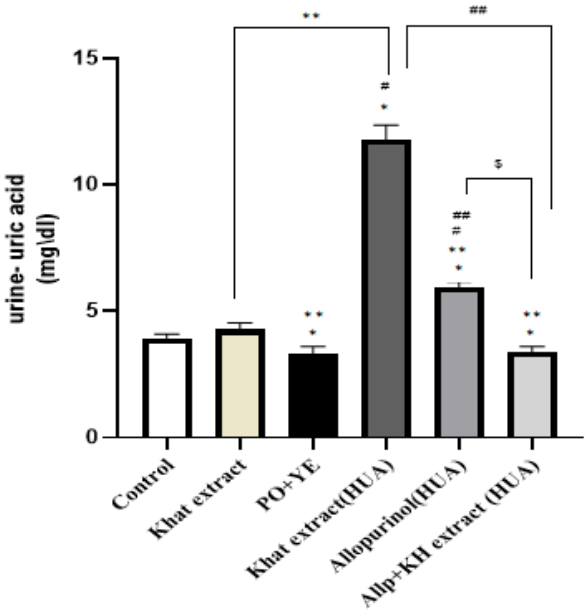

Figure 3. Urine uric acid levels (mg/dl) in different experimental groups (n = 8 per group).

3.5. Effects of the Khat Extract and Allopurinol on Xanthine Oxidase Activity

As shown in Figure (4), serum uric acid levels were significantly higher in PO+YE, khat extract–HUA, allopurinol, and allopurinol plus khat extract groups than in the control ($p < 0.0001$ for all except khat extract, $p = 0.0023$). Compared with the khat extract group, PO+YE, khat extract–HUA, and allopurinol plus khat extract groups showed significantly higher levels ($p < 0.0001$), whereas no difference was observed between khat extract and allopurinol. The khat extract–HUA group consistently exhibited the highest levels, which were significantly greater than those in PO+YE and allopurinol ($p < 0.0001$). Allopurinol alone resulted in lower levels than the combination with khat extract ($p < 0.0001$).

Values are expressed as the mean ± standard deviation. PO+YE: potassium oxonate plus yeast extract. Khat extract (HUA): khat extract under hyperuricemia conditions. Allp+Khat extract (HUA): allopurinol with khat extract under hyperuricemia conditions

* p = 0.05 vs. Control,

** p = 0.05 vs. Khat extract,

p = 0.05 vs. PO+YE,

p = 0.05 vs. Khat extract-HUA,

$ p = 0.05 vs. Allopurinol + Khat extract)HUA)

3.6. Effects of Khat Extract and Allopurinol on Renal Functions

As shown in Table (2), serum creatinine and BUN levels were significantly different among the groups, with no difference between some pairs (PO+YE vs. Khat extract-

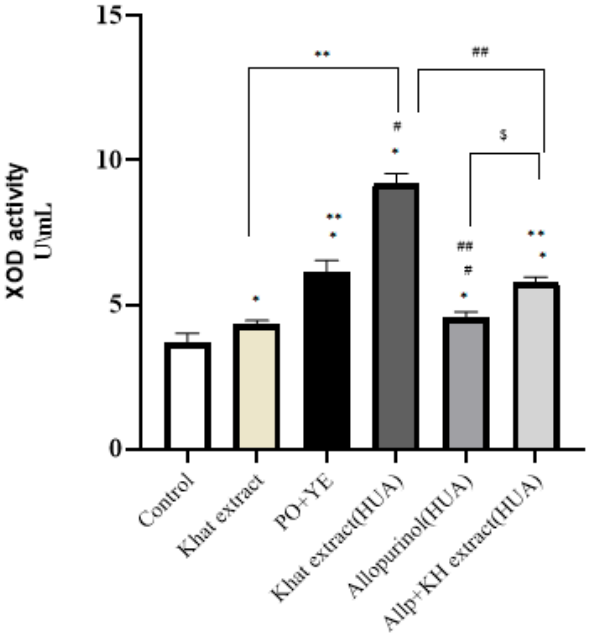

Figure 4. Xanthine oxidase activity levels (XOD-U/mL) in different experimental groups (n = 8 per group).

HUA; allopurinol vs. allopurinol+Khat extract). Compared to those in the control group, albumin levels in the Khat extract, PO+YE, Khat extract-HUA, and allopurinol+Khat extract groups were significantly lower. The allopurinol+Khat extract group also showed a greater decrease than the allopurinol group. Total protein levels differed among the groups, but no significant differences were found in specific pairs (e.g., PO+YE vs. Khat extract-HUA).

3.7. Liver function test (ALT, AST, ALP, and total bilirubin)

As shown in Table (3), ALT levels were significantly higher in the Khat extract (P=0.008), PO+YE (P=0.001), and Khat extract-HUA (P=0.04) groups than in the control group. AST levels were significantly lower in the allopurinol (P=0.006) and allopurinol+Khat extract (P=0.003) groups.ALP activity was markedly elevated in the PO+YE, Khat extract-HUA (P=0.001), and allopurinol+Khat extract groups (P=0.001), whereas the level of allopurinol was markedly increased, as were the intergroup differences (P=0.019–0.001). Total bilirubin increased only in the allopurinol+Khat extract group (P=0.04) but decreased with allopurinol alone (P=0.016).

3.8. Effects of khat extract and allopurinol on CBC

As shown in Table (4-5), the hematological parameters differed significantly among the groups. Hb and PCV: Compared with the control, both allopurinol (P=0.017, 0.002) and allopurinol+Khat extract (P=0.001) markedly decreased Hb and PCV. WBC counts were elevated in the Khat extract (P=0.001, 0.035) and Khat extract-HUA groups (P=0.001) but decreased in the PO+YE group (P=0.001) compared with the control group.

Neutrophils/Lymphocytes: Khat extract-HUA resulted in more neutrophils (P=0.002) and fewer lymphocytes (P=0.03, 0.005) than PO+YE, which increased the number of lymphocytes (P=0.026, 0.014).

Platelets: Platelet count measurements were significantly different among the studied groups ($P \leq 0.001$).

3.9. Effects of khat extract and allopurinol on urine analysis

As shown in Table (6), urine analysis revealed marked changes across the groups. The control group had clear urine (100%), acidic pH (100%), and no nitrites, crystals, or bacteria. In contrast, the Khat extract group was highly positive for nitrites (50%), Ca-oxalate crystals (80%), and bacteria (50%), with only 25% clarity. The PO+YE and Khat extract-HUA groups also presented high percentages of nitrites (85% and 14%) and crystals (50% and 72%), respectively. The allopurinol and allopurinol plus Khat extract groups presented moderate abnormalities with reduced clarity (29% and 20%) and the presence of nitrites, crystals, and bacteria, as shown in Table (6).

3.10. Histological analysis

a. Kidney histopathology

Photomicrographs of kidney tissue stained with hematoxylin and eosin from different rat experimental groups. As shown in Figure (5), sections from the control group (G1) presented a normal renal cortex with normal glomeruli, Bowman's capsules (green arrows), proximal convoluted tubules (red arrows), and distal convoluted tubules (yellow arrows). [400x, scale bar=50 μm].

As shown in Figure (5), the sections of the Khat-extract group (G2) revealed a normal renal cortex with normal glomeruli and Bowman's capsule (green arrows), proximal convoluted tubules (red arrows), and distal convoluted tubules (yellow arrows). [400x scale bar=50 μm].

As shown in Figure (5), the sections of the PO+YE group (G3b) revealed focal tubular injury. Some tubules were filled with necrotic epithelial debris and urate crystals (white arrows), while others were filled with eosinophilic hyaline casts (arrowheads). Tubular vacuolation was also observed in some tubules (black arrow), and congestion of capillaries (red arrows) was also observed. [400x scale bar=50 μm].

As shown in Figure (6), the khat extract (HUA) group G4 (G4a, G4b, G4c, and G4d) sections revealed prominent tubular and interstitial injury. Most tubules (blue arrows) were widened and distorted. Some of them were filled with necrotic epithelial debris and urate crystals (white arrows), and some were filled with eosinophilic

Table[2]: Effects of khat extract and allopurinol on kidney function

Group	Mean ±SD			
	Creatinine (mg/dl)	BUN (mg/dl)	Albumin (mg/dl)	Total Protein (mg/dl)
Control	0.30±0.01	4.4±0.1	36±1.1	756±20
Khat extract	0.73±0.02*	7±0.2*	33±1.7*	686±19 *
PO+YE	1.3±0.08 *,**	16.9±0.3 *,**	29±1.3*,**	527±6.5*,**
Khat extract-HUA	1.4± 0.09*,**	18.8±0.91*,**,#	29±1.4*,**	523±7.4 *,**
Allopurinol	0.93±0.05*,**,#,##	9.9±0.7*,**#,##	35±1.6#,##	641±5.3*,**#,##
Khat extract plus Allopurinol	1±0.06*,**,#,##	12.2±0.4*,**#,##,^	28±0.9*,**,$	679±8 *#,##,$

BUN= blood urea nitrogen; PO+YE= potassium oxonate plus yeast extract; Khat (HUA): khat extract under hyperuricemia conditions.
*=significant difference from the control, p value ≤0.001 **=significant difference from Khat extract, p value ≤0.001
#=significant difference from PO+YE p value ≤0.001 ##=significant difference from Khat extract-HUA p value ≤0.001
$=significant difference from allopurinol, p value ≤0.001

Table[3]: Effects of khat extract and allopurinol on ALT, AST, ALP, and Total bilirubin

Groups	Mean ±SD			
	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)
Control	40±1.2	82.3±5.6	155±6	0.5±0.24
Khat extract	46±5.7 *	84.2±5.3	191±17*	0.6±0.24
PO+YE	49±3.4*	91±6.2	201±9*	0.7±0.36
Khat extract-HUA	44±2.0*,#	82±13.1	203±14 *	0.74±0.45
Allopurinol	44±4.8	75±8 #	173±14 *,**,#,##	0.4±0.33
Khat extract plus Allopurinol	46.1±3.1 *	75.1±8.5 #	187±10 *, #,##	0.9±0.49 *,$

Table[4]: Effects of khat extract and allopurinol on Hb, P.CV, and platelet counts

Group	Mean ±SD		
	Hb (g\dl)	P.C.V (%)	Platelet count (×109/L)
Control	17.3±1.2	51.9±1.7	539±2
KH extract	17.7±0.7	53.4±1.6	663±8*
PO+YE	17.3±0.7	54.6±41	485±6*,**
KH-extract-HUA	18.5±1	52.9±3.0	720±7.7 *,**,#
Allopurinol	14.1±2.6**,##	44.6±5.7#	693±4 *,**,#,##
Khat extract plus Allopurinol	14.2±1.7*,**,#,##	45±6.2**, #	722±8*,**,#,##,$

PO+YE: potassium oxonate plus yeast extract; Khat (HUA): khat extract under hyperuricemia conditions; Hb: hemoglobin, PCV: packed cell volume.
*=significant difference from the control, p value ≤0.05 **=significant difference from Khat extract, p value ≤0.05,
#=significant difference from PO+YE, p value ≤0.05 ##=significant difference from Khat extract-HUA, p value ≤0.05,
$=significant difference from allopurinol plus Khat extract p value ≤0.05

hyaline casts (arrowheads). Tubular vacuolation was also observed in some tubules (black arrow). A marked inflammatory reaction was observed in the interstitium (stars), which was composed of a mixture of inflammatory cells, such as eosinophil, and lymphocytes. In areas of severe tubulointerstitial inflammation, tubules were lost and replaced by inflammatory cells and fibrosis (circles). [all 400x, scale bar=50 μm]

As shown in Figure (6), renal histology noticeably improved in the allopurinol group (G5). Most glomeruli

Table[5]: Effects of khat extract and allopurinol on W.B.C, Neutrophils, and lymphocytes

Group	Mean ±SD		
	W.B.C (×109/L)	Neutrophils (%)	Lymphocytes (%)
Control	10.2±1.9	50.2±4.88	32.2±5.17
KH extract	13.1±37 #,##	51.2±8.54	30.5±6.44
PO+YE	6± 1.45 ##	50.3±4.7##	42.8±6.8*,**
KH-extract-HUA	16.6±17*,**	34.3±7.5*,**	37.4±6.96
Allopurinol	9.4±2.4 #,##	46.9±9.7*,**#	40.4±7.7*,**
Khat extract plus Allopurinol	10.3±42 #,##	43.6±11.7 #	37.5±4.8*,*

PO+YE: potassium oxonate plus yeast extract; Khat (HUA): khat extract under hyperuricemia conditions; WBC: white blood cell.
*=significant difference from the control, p value ≤0.05 **=significant difference from Khat extract, p value ≤0.05,
#=significant difference from PO+YE, p value ≤0.05 ##=significant difference from Khat extract-HUA, p value ≤0.05,
$=significant difference from allopurinol plus Khat extract p value ≤0.05

Table[6]: Effects of khat extract and allopurinol on urine analysis.

Parameter	Clarity (%)	pH (%)	Nitrites (%)	Ca-Oxalate (%)	Bacteria (%)
	Clear	Acid	+	+	+
Control	100	100	0	0	0
Khat extract	25	75	50	80	50
PO+YE	57	100	85	50	57
Khat extract-HUA	14	72	14	72	10
Allopurinol	29	57	43	71	42
Allopurinol plus Khat extract	20	86	50	50	40

PO+YE: potassium oxonate plus yeast extract; Khat extract (HUA): khat extract under hyperuricemia conditions

(green arrows), proximal convoluted tubules (red arrows), and distal convoluted tubules (yellow arrows) had a normal histological appearance. [400x, scale bar=50 μm]

As shown in Figure (6), the sections of the allopurinol plus khat extract group (G6) revealed some tubular and interstitial injury. Some tubules (blue arrows) were widened, distorted, and filled with necrotic epithelial debris and urate crystals (white arrows), and a few tubules were filled with eosinophilic, hyaline casts (arrowheads). Tubular vacuolation was also observed in some tubules (black arrows). A moderate degree of inflammatory reaction was observed in the interstitium mixed with fibrosis (stars) [all 400x, scale bar=50 μm].

b. Liver histopathology (all stained with PAS)

Figure (7) shows photomicrographs of PAS-stained liver tissues from different experimental groups of rats. Sections from the control group (G1) showed normal hepatic parenchyma with well-organized radiating arrangements of hepatic cords and sinusoids (yellow arrows) around the central vein (CV). Hepatocytes (white arrows) were uniform and had large nuclei with prominent nucleoli and normal glycogen content. No signs of inflammation, necrosis, fatty changes, or vacuolization were noted. [400x, scale bar=50 μm]

As shown in Figure (7), the sections of the Khat-extract group (G2) showed congestion of the central vein (CV). Mild widening and congestion of some sinusoids compared with normal sinusoids (red arrows). Hepatocytes (white arrows) were uniform and had large nuclei with prominent nucleoli and normal glycogen content. No signs of inflammation, necrotic changes, fatty changes, or vacuolization were noted. [400x, scale bar=50 μm]

As shown in Figure (7), rats in groups G3a and G3b were administered a combination of potassium oxonate and yeast extract. Liver sections showed diffuse ballooning of hepatocytes (arrowheads) with depletion of glycogen content and focal collections of inflammatory cells (blue arrows). [100x, 400x; scale bar=50 μm].

Figure (7): Histopathology of liver tissue from the control (G1), Khat extract (G2) and PO plus YE (G3a -3b) groups . PAS stain [100x -400x, scale bar=50 μm]

As shown in Figure (8), in the Khat extract (HUA) group (G4a, G4b), liver sections showed widening and congestion of the central vein (CV), diffuse ballooning of hepatocytes (arrowheads) with depletion of glycogen content, and focal collections of inflammatory cells (blue arrows), mainly in the portal areas. [100x, 400x; scale bar=50 μm].

As shown in Figure (8), compared with the Khat extract (HUA) group, the liver histology of the allopurinol group (G5a and G5b) was mildly improved. However, pathological changes, such as widening and congestion of the central vein (CV), ballooning of hepatocytes (arrowheads), and focal collection of inflammatory cells (blue arrows), persisted in some sections. Most hepatocytes had normal glycogen content. [100x, 400x; scale bar=50

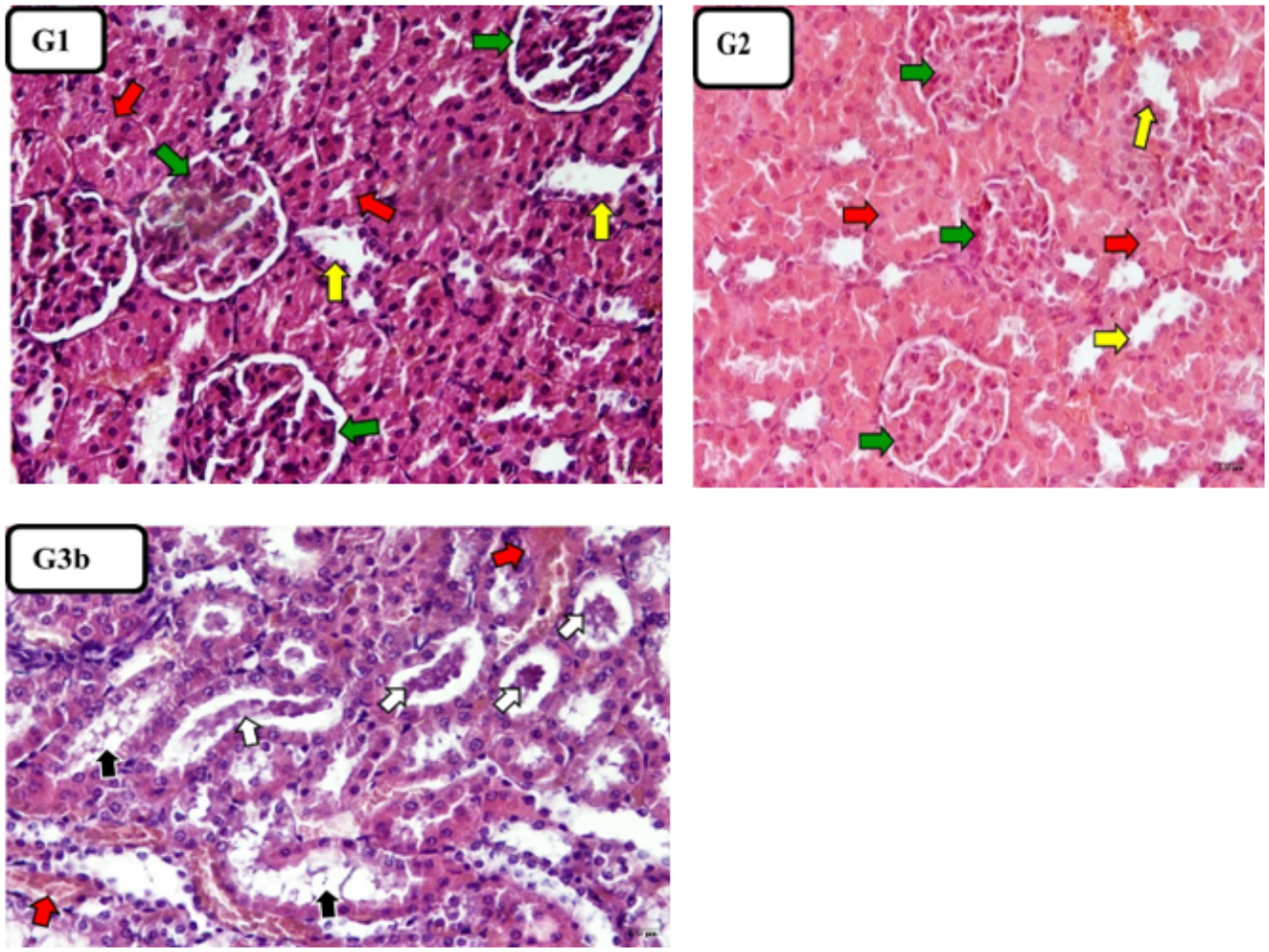

Figure 5. Histopathology of kidney tissue from the control (G1), Khat-extract (G2), PO+YE (G3b). H&E staining [400x, scale bar=50 μm]

μm].

As shown in Figure (8), compared with those of the PO+YE and Khat extract-HUA groups, the liver histology of the allopurinol plus Khat extract-G6 group improved. Hepatocytes have normal glycogen content. However, the central vein (CV) still showed mild widening, and the hepatocytes presented with cytoplasmic microvesicles (arrowheads). [400x, scale bar=50 μm].

4. DISCUSSION

Serum uric acid levels were significantly elevated in PO+YE rats, confirming successful hyperuricemia induction, consistent with previous studies [20].

Khat extract (HUA) and PO+YE groups showed higher serum uric acid levels than the allopurinol-treated groups, consistent with animal studies reporting khat-induced uric acid elevation [21, 22]. Similarly, human clinical trials have shown that khat chewers have significantly higher uric acid levels than non-chewers, although these levels remain within the normal range [23–26]. These findings indicate that khat extract, either alone or in combination with allopurinol, enhances uric acid production and reduces the therapeutic effectiveness of allopurinol, suggesting a potential pharmacological interaction.

Compared with those in the other groups, the urinary uric acid levels in the HUA group were significantly higher. Compared with PO, allopurinol markedly increased urinary urate levels and renal urate excretion in rats [27, 28]. Another study demonstrated that the standard drug allopurinol enhanced urinary uric acid excretion compared with that of the normal control but reduced it compared with that of PO-induced rats [29].

Compared with the other groups, the extract-HUA group showed a significant increase in xanthine oxidase activity. The administration of khat enhanced xanthine oxidase activity, leading to an increase in uric acid levels in the subjects [30]. Allopurinol had significant inhibitory effects on serum XOD levels in rats [27, 31].

Khat extract impaired renal function, shown by increased creatinine and BUN and reduced albumin and total protein, consistent with reported nephrotoxic effects [32–35]. Although they contradict the findings of Othman et al. (2024), who reported increased albumin and total protein levels in khat chewers [36]. Additionally, both the PO and allopurinol groups presented elevated creatinine

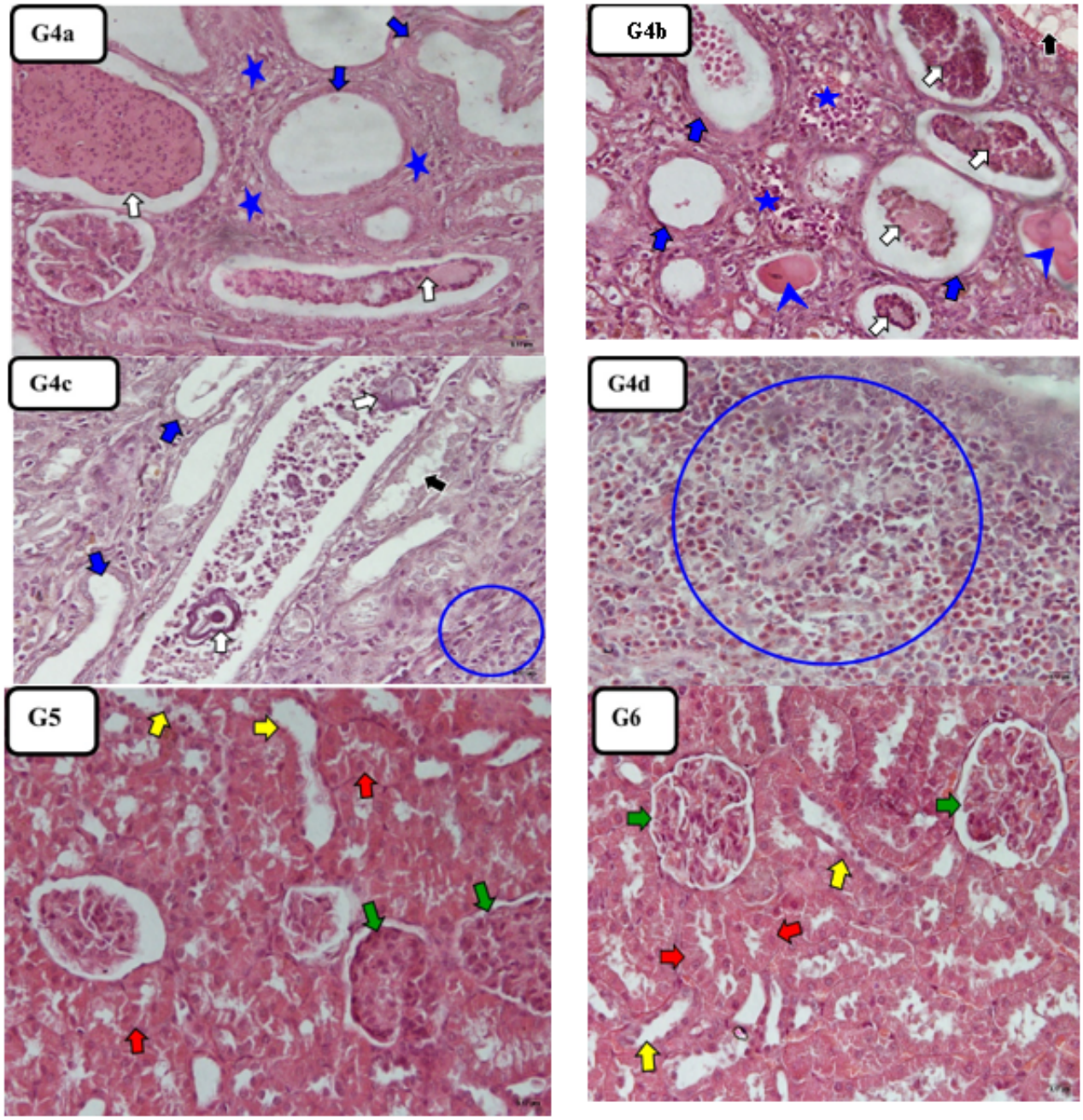

Figure 6. Histopathology of kidney tissue from the Khat-extract-HUA (G4a, b, c, d), allopurinol (G5) and allopurinol+ Khat-extract (G6) groups. H&E staining [400x, scale bar=50 μm]

and BUN levels [27]. While some animal studies have suggested that allopurinol exerts neither protective nor harmful renal effects [37]. Coadministration of allopurinol with khat extract aggravated renal dysfunction and histological damage, indicating additive toxicity, unlike clinical evidence showing renal benefits of allopurinol [38–40].

Khat extract significantly altered liver function markers, with variations in ALT, ALP, and AST levels between the groups, whereas total bilirubin levels remained unchanged. These findings support earlier evidence, as Alsalahi et al. reported abnormalities in AST and ALP levels following khat exposure in animal models [41], and clinical investigations have documented hepatic injury with elevated liver enzymes among khat chewers [42]. The hepatoprotective role of allopurinol, as shown in rabbits [43], was also reflected in the present study.

Urinalysis indicated adverse urinary changes with khat extract, including altered urine characteristics and an increased infection risk. These outcomes are consistent with clinical reports linking khat chewing with urinary tract symptoms [44] and with other studies identifying khat chewing as a risk factor for urinary tract infection in comorbid conditions [45].

The current study showed that hemoglobin levels were reduced in the allopurinol and allopurinol plus khat extract groups, while MCH and MCHC were significantly

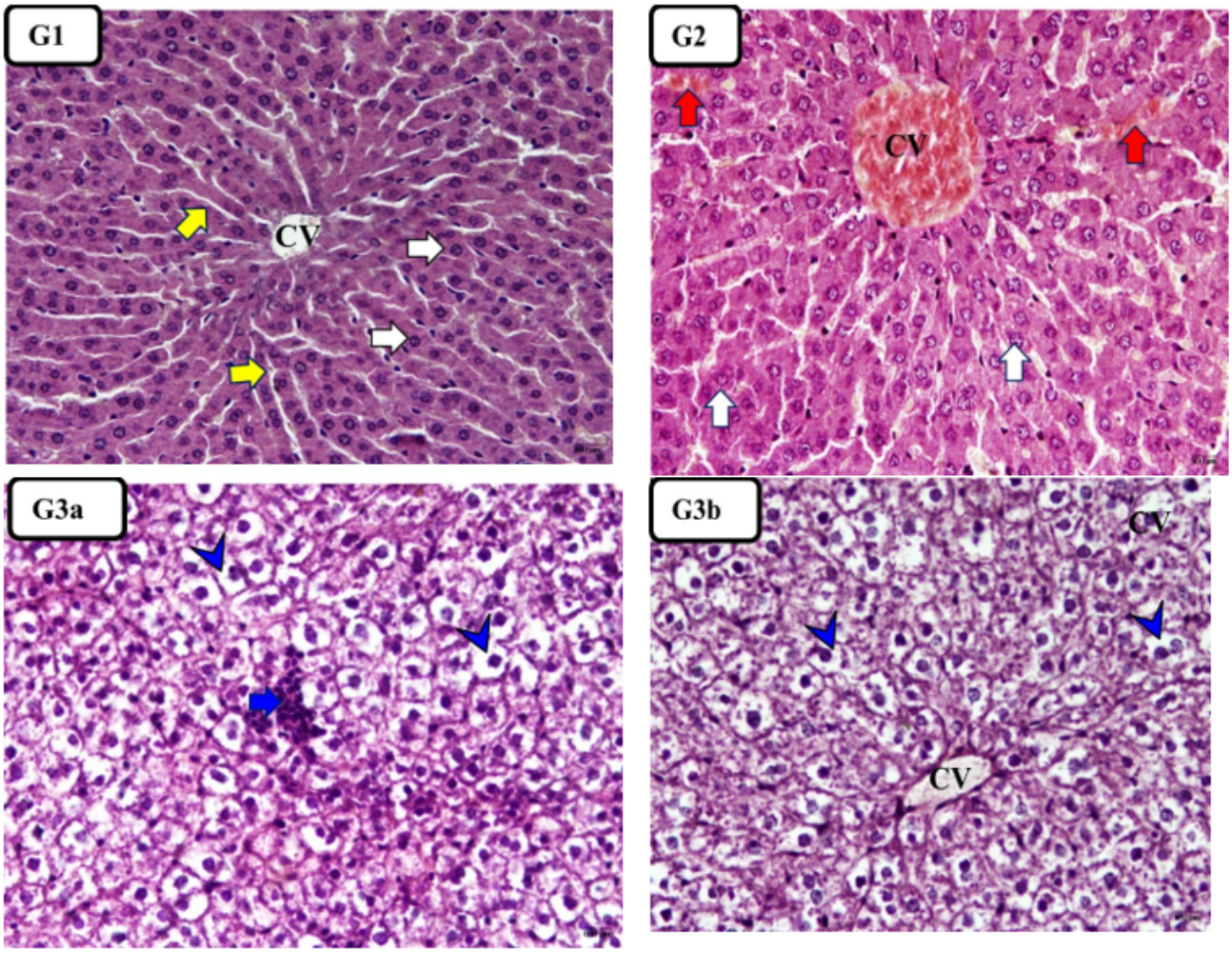

Figure 7. Histopathology of liver tissue from control (G1), khat extract (G2), and PO+YE (G3a,b). PAS stain [100x -400x, scale bar=50 μm]

higher in the khat plus HUA group. These findings align with previous clinical [46] and preclinical studies [47, 48] that documented hematological disturbances associated with prolonged khat consumption. However, conflicting evidence exists, as one study reported no significant differences in CBC parameters between *Catha edulis* and treated rats and controls [49]. Such discrepancies may reflect differences in the experimental design, duration of exposure, or dosing regimens.

Severe kidney damage was observed in the PO+YE and khat groups, whereas allopurinol alone maintained normal kidney structure. However, when combined with khat, allopurinol did not protect the kidneys. Similar studies have shown that khat can cause kidney inflammation, enlargement of glomerular capillaries, and widening of Bowman's capsules [32, 34, 35].

Conversely, most studies reported an absence of significant histopathological changes in the kidneys of rats treated with allopurinol alone [31, 37], which is consistent with the present results.

Liver histology showed congestion and hepatocellular degeneration in the PO+YE and khat groups, with only mild improvement by allopurinol and persistent changes when combined with khat, consistent with previous reports [50]. Furthermore, histological analysis of livers from allopurinol-treated rats revealed minimal necrosis, mild fatty infiltration, and congestion [43], supporting the current findings.

Overall, the findings indicate that khat extract negatively influences the hypouricemic and organ-protective effects of allopurinol, emphasizing the need for caution when using herbal products in conjunction with conventional therapies.

5. CONCLUSION

This study demonstrated that khat extract, whether administered alone or in combination with allopurinol, worsened hyperuricemia in rats by increasing serum uric acid levels and xanthine oxidase activity, impairing renal and hepatic function, and causing marked histopathological damage. Co-administration of khat with allopurinol reduced the hypouricemic effect of allopurinol and further exacerbated the biochemical and tissue alterations. These findings highlight the potential risks of concurrent

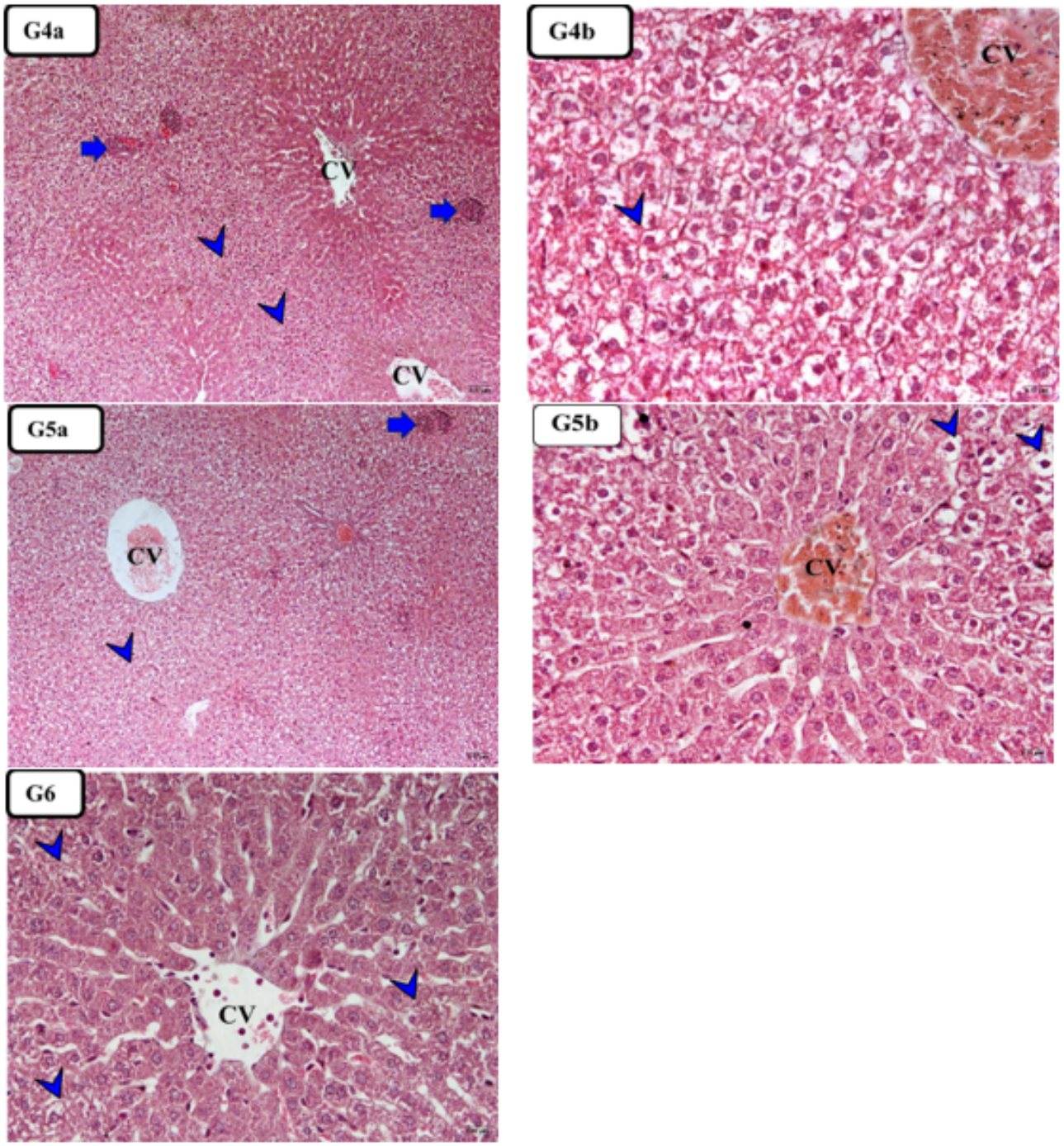

Figure 8. Histopathology of liver tissue from the Khat extract (HUA) (G4a-b), allopurinol (G5a-b), and allopurinol plus khat extract (G6) groups. PAS stain [100x -400x, scale bar=50 μm]

khat use during allopurinol therapy in this animal model and underscore the need for further studies to confirm these effects in humans.

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