



## Effect of Xylazine Administration on Ketoprofen Toxicity in Mice

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**T**HE goal of our study was to assess the influence of xylazine on the toxicity of ketoprofen in mice, then we determined the effect of co-administration of xylazine and ketoprofen on neurobehavioral toxicity and liver and kidney function as well as histopathological effects after repeated daily administration for 20 days. The LD<sub>50</sub> of ketoprofen & xylazine were respectively (375mg/kg, 75 mg/kg) respectively. The injection of ketoprofen alone at a dose of (0.126 mg/kg) & ketoprofen & xylazine together at a dose of (0.663 mg/kg) caused the crossed squares & rearing to decline significantly. This was accompanied by a significant increase in the time it took the mouse to return to its normal position and a significant decline in the frequency of head poking. On the tenth and twenty-fifth day of administration of ketoprofen at (71.95 mg/kg) & xylazine at (14.156 mg/kg i.p) there was a significant elevation in the concentration of liver enzymes (AST & ALT) & kidney function (Creatinine and uric acid), as well as causing histological changes in the liver, kidney & brain. Our findings reveal that the xylazine caused an exacerbation of ketoprofen toxicity which appear as a depressant effect on the CNS, liver and kidney, furthermore histopathological changes in the liver, kidney, and brain.

**Keywords:** Ketoprofen, Xylazine, Toxicity, Mice.

### Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a type of medication that is used to treat pain and inflammation with a number of inflammatory disorders like musculoskeletal injuries, gout, rheumatoid arthritis, and osteoarthritis [1]. Despite the fact that (NSAIDs) treatment is effective, but it has unfavorable liver injury, an elevated risk of mucosal damage, and fatalities in both the upper & lower gastrointestinal tracts are also present [2], as well as potential nephrotoxic effect [3,4].

Other adverse effects associated with NSAIDs were renal, electrolyte retention, glomerular filtration [5]. Hyperkalemia, Nephrotic Syndrome, and Cardiovascular effects such as increased Blood Pressure, chest pain from congestive heart failure, thrombotic or failure effects [6]. The (NSAIDs) family includes ketoprofen with analgesic and

antipyretic properties [7,8]. Ketoprofen may reduce the swelling that skin tumors experience when they inflame. A condition that is becoming more prevalent. The peri-tumoral inflammatory response in these tumors serves as a protective mechanism while also promoting the neoplastic process [9]. These compounds (NSAIDs) act as pharmacological stomach antibiotics, anti-inflammatory agents, and anti-secretory agents, they are frequently used to treat postoperative pain [10]. Its use involves the administration of antihistamine H<sub>2</sub>, which are medications that, when combined with a last-generation cephalosporin or long-term antibiotherapy, might increase the risk of Clostridium difficile infection, particularly if surgical colon disease is present [11]. The biliary elimination and anticoagulant activity of NSAIDs should directly correlate with their effects on the stomach, kidney, and liver [12,13].

According to other studies xylazine slows both the central and peripheral neurological systems. The nervous systems of the brain and spinal cord are both reportedly slowed down by xylazine. The central and peripheral nervous systems' production of noradrenaline is inhibited by stimulating the  $\alpha$ -2-adrenergic receptors found in the pre-synaptic terminals of neurons. This decreases calcium entry, which in turn inhibits noradrenaline secretion [14-16].

Our study's objective was to assess the toxicity of ketoprofen alone and in the presence of xylazine in mice through locomotor activity and neurobehavioral experiments, measuring liver enzyme activity and kidney function, as well as histopathological tests of the brain, liver, and kidney.

## **Material and Methods**

### *Ethical approval*

We acquired formal clearance for the study design from the Committee of Postgraduate Studies at the Faculty of Veterinary Medicine, University of Mosul, Iraq, in accordance with institutional policy on animal care and utilization in research. UM.VET.2021.064

### *Animals*

In this study, 101 Swiss albino male and female mice were used. They were equipped by the Animal House, College of Veterinary Medicine, and University of Mosul. Their weights ranged between 25-38 g. Their ages ranged between 2-3 months. The convergence of weights and ages was taken into account within one experiment.

### *Drugs*

Ketoprofen 10% is manufactured by NITA-FARM, Russia. Xylazine 2% produced by Intercheme, Holland. Physiological salt solution POLIFARMA company, Turkey.

## **Experiments**

### *Determination of the LD50 of ketoprofen and xylazine using the Up and down procedure.*

According to the previously reported up-and-down approach, the acute LD50 of xylazine and ketoprofen were measured [17-19].

The first intra-peritoneal (IP) dose of ketoprofen was 500 mg/kg. While the first dose of xylazine (IP) was 100mg/kg. After 24 hours, the results were recorded as (death X or life O), and the dosage adjustments for xylazine (25 mg/kg) and ketoprofen (125 mg/kg) were consistent. To

determine the LD<sub>50</sub> using Dixon's equation and illustration, repeat dosing up or down for 3 mice after changing the outcome from death to life, and vice versa [17].

### *Neurobehavioral effects of repeated daily dosage of ketoprofen & xylazine*

Three groups of five mice each were formed out of fifteen animals. The control group received an injection of normal saline. The second group received a 0.126 mg/kg B.Wt. injection of ketoprofen. The third group received ketoprofen 0,126 and xylazine at 0.663 mg/kg B.Wt. After 5,10 and 20 days of intra-peritoneal injected of ketoprofen and xylazine, all mice subjected to neurobehavioral tests:

**Open - field:** Mice were treated to the open-field test utilizing a 35x35x25 cm box, the arena of the test, which assesses locomotor behavior. Each mouse was put in the center of the arena and instructed to measure the number of lines crossed between its four legs, the number of rearing, and defecation, within 3 minutes. The box was divided into 25 identical squares [20].

**Negative geotaxis:** This test assesses neuro-motor coordination, vestibular action, and the related integrative motor response to space orientation. This behavior was assessed on a sloped, rough wooden surface at an angle of 45°, and each mouse's performance was timed for 180° turns, with a 60-second maximum time limit. [21]. A plastic circular surface with a diameter of 30 cm, low edges, and a height of 10 cm, and 8 holes spaced 2 cm apart is used for the head-pocking test, which measures the locomotion, curiosity, and movement of the treated mouse. The mouse is placed on the surface for three minutes while being observed for head-pocking in the holes [22]. The swimming test involves dropping a mouse into a glass tank filled with water that is 29 to 30 degrees Celsius in temperature and 30 cm deep for 5 to 10 seconds. Scores are recorded while the mouse is being closely observed and how the treated mouse responds to the stressful setting [23].

### *Effects of ketoprofen alone or ketoprofen and xylazine on the liver and kidney function and histopathological effects in mice.*

Forty five mice were divided randomly into three groups in each group 15 mice were injected into the first group with physiological saline solution, the second group was injected with ketoprofen at a dose of 71.95 mg/kg IP., and the third group was injected with ketoprofen at a

dose of 71.95 mg/kg and xylazine at a dose of 14.156 mg/kg for 20 days. On the fifth, tenth and twentieth day of the injection, the mice were scarified and blood samples were drawn to measure AST and ALT, creatinine, uric acid, and the liver, kidney and brain were taken to examination histological sections. An incision was made, and 1 cm of liver, kidney and brain were harvested. For 72 hours, Tissue samples of the liver, kidney and brain were taken and preserved in 10% formalin for histopathological examination. Fixed tissues were processed in accordance with the accepted histological procedure, and hematoxylin and eosin was used to stain thick slices measuring 5 to 6 m. [24]. Images were captured using a digital camera while sections were inspected under a light microscope (HDCM-5)

#### Statistical analysis

One-way analysis of variance (ANOVA) test version 16.0 was used to statistically examine the parametric data, and the least significant difference test was applied using the statistical analysis software SPSS. The level of significance was set at P0.05 when the non-parametric findings were statistically examined using the mann-whitney U test [25].

## Results

The LD<sub>50</sub> of ketoprofen in mice was 375 mg/kg IP. The mice treated with ketoprofen showed signs of poisoning within 2-5 minutes. They were as follows: lethargy, hair erection, slow breathing, and writhing. Table 1. While the LD<sub>50</sub> of xylazine was 75 mg / kg IP., and the mice treated with xylazine showed signs of poisoning within 2-10 minutes, and the signs of poisoning were lethargy, hair erection, and then death Table 2.

#### Neurobehavioral effects of repeated doses of ketoprofen and xylazine in mice

Motor activity test in the open field: the injection of ketoprofen alone at a dose of 0.126 mg / kg & ketoprofen 0.126 mg/kg i.p. & xylazine together at a dose of 0.663 mg/kg in the fifth, tenth & twentieth day caused a significant decline in the number of line cross and number of standing within three minutes in comparison to the control group (Table 3).

In the negative geotaxis test: we note no significant change in the time of the mouse's return to its normal position in the group treated with ketoprofen at a dose of 0.126 mg / kg i.p., in the fifth, tenth while the injection of ketoprofen with xylazine at a dose of 0.663 mg / kg.i.p.

**TABLE 1. The acute median lethal dose (LD50) of ketoprofen (IP) by Dixon method**

LD <sub>50</sub> of ketoprofen	375 mg/kg IP
Range of dose	500-250=250
The first dose	500
The end dose	250
Raise and reduction in the dose	125
The number of chicks	5 (XOXXO)

O: live mice X: death of mice

**TABLE 2. The acute median lethal dose (LD<sub>50</sub>) of xylazine IP by Dixon method**

LD <sub>50</sub> of xylazine	75 mg/kg i.p.
Range of dose	100-50=50
The first dose	100
The end dose	50
Raise and reduction in the dose	25
The number of chicks	6 (XXOXOO)

O: live mice X: death of mice

**TABLE 3. The effects of ketoprofen alone or with xylazine during 5,10 and 20 days on the levels of mice motor and neurobehavioral activities**

Days	Parameters	Groups		
		Control	Ketoprofen	Ketoprofen + xylazine
5	Negative Geotaxis	4.60 ± 0.78	4.80 ± 1.36	5.80 ± 1.32*
	Head Pocking	15.60 ± 1.21	8.20 ± 2.85*	6.20 ± 3.48*.a
	Swimming	4.00 ± 0.00	4.00 ± 0.00	3.80 ± 0.20
	Square Number	106.40 ± 23.57	76.20 ± 11.69*	52.40 ± 11.01*.a
	Rearing	15.60 ± 1.21	8.20 ± 2.85*	6.20 ± 3.48*.a
	defecation	1.60 ± 0.60	1.80 ± 0.20*	3.20 ± 0.49*.a
10	Negative Geotaxis	5.60 ± 1.47	8.80 ± 3.26	9.80 ± 3.63*
	Head Pocking	13.00 ± 0.63	10.80 ± 0.58	9.00 ± 2.68*
	Swimming	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	Square Number	87.20 ± 12.72	69.60 ± 16.28*	44.00 ± 11.66*.a
	rearing	13.00 ± 0.63	10.80 ± 0.58*	9.00 ± 2.68*
	defecation	1.20 ± 0.91	1.40 ± 0.61	1.60 ± 0.51*
20	Geotaxis Negative	8.00 ± 1.92	12.60 ± 3.59*	23.80 ± 6.58*.a
	Head Pocking	9.00 ± 2.51	7.20 ± 2.15*	5.40 ± 1.29*.a
	Swimming	4.00 ± 0.00	3.60 ± 0.24	3.20 ± 0.20*
	Square Number	79.80 ± 13.66	61.00 ± 13.99*	51.60 ± 14.85*.a
	Rearing	9.00 ± 2.51	7.20 ± 2.15*	5.40 ± 1.29*.a
	Defecation	2.40 ± 0.51	2.40 ± 0.81	2.20 ± 0.66

The values represented mean ±SE for 5 mice/group. Ketamine was injected alone (0,126mg/kg, IP) or with xylazine (0.663 mg/kg, IP).

(\*): Significantly change with the control group at  $P \leq 0.05$

(a): Significantly change with the ketamine alone group at  $P \leq 0.05$ .

Caused a significant elevated in the return time in comparison to the control group in the fifth, tenth and twentieth day (Table 3).

- In the pocking of the head test: the injection of ketoprofen alone at a dose of 0.126 mg / kg i.p. and ketoprofen at a dose 0.126mg/kg with xylazine at a dose of 0.663 caused a significant decline in the number of times the head was inserted in both (within 3 minutes) in comparison to the control group in the fifth and twentieth day (Table 3).
- In the swimming test, there was no significant difference between the ketoprofen group alone and the ketoprofen and xylazine group together, in comparison to the control group (Table: 3).

Injection of ketoprofen alone at a dose 71.95mg/kg IP, and ketoprofen and xylazine (71.95 + 14.156 mg/kg IP) together on the fifth day of injection caused a significant increase in the level of creatinine and uric acid, accompanied

by a significant increase in the level of AST enzyme, while the administration of ketoprofen and xylazine together on the tenth day caused a significant increase in the both level of liver enzymes (AST and ALT) compared with the control group (Table 4). On the twentieth day of injection of ketoprofen alone in a dose 71.95 mg/kg IP. that caused a significant increase in the level of uric acid, while the injection of ketoprofen and xylazine (71.95+14.156mg/kg) together caused a significant increase in the level of creatinine, uric acid and AST, ALT, compared with the control group (Table 4).

#### *Pathological finding*

*Brain:* ketoprofen at a dose of 71.95 mg / kg and IP. After the fifth and twenty-tenth day of injection showed histological changes in the brain, including: congestion of the meningeal blood vessels, vasogenic edema, cellular edema, necrosis of neuronal cells, increase of inflammatory cells (Fig. 2, 4, 6). While Ketoprofen

**TABLE 4. The effects of ketoprofen injection and/ or with xylazine after 5,10 and 20 days of injection on some biochemical values of liver and kidney function**

Days	Parameters	Groups		
		Control	ketoprofen	ketoprofen + xylazine
5	Creatinine	0.49 ± 0.02	1.27 ± 0.50*	1.52 ± 0.57 <sup>*a</sup>
	Uric acid	1.00 ± 0.50	2.73 ± 1.25*	4.40 ± 0.50 <sup>*a</sup>
	AST	103.10 ± 11.52	152.20 ± 19.91*	192 ± 25.84 <sup>*a</sup>
	ALT	56.50 ± 5.21	60.47 ± 15.23	79.80 ± 9.63*
10	Creatinine	0.46 ± 0.05	0.57 ± 0.07	0.59 ± 0.14
	Uric acid	2.26 ± 0.67	2.28 ± 0.55	3.34 ± 0.73*
	AST	122.92 ± 16.14	152.82 ± 17.88*	156.54 ± 21.24 <sup>*a</sup>
	ALT	63.34 ± 16.98	82.52 ± 10.24*	118.56 ± 42.18 <sup>*a</sup>
20	Creatinine	0.48 ± 0.10	0.51 ± 0.04	0.60 ± 0.05*
	Uric acid	1.00 ± 0.14	1.93 ± 0.34*	2.12 ± 0.35 <sup>*a</sup>
	AST	93.28 ± 10.16	100.13 ± 26.56	102.38 ± 6.43*
	ALT	42.55 ± 7.47	43.30 ± 4.65	47.35 ± 3.41*

The values represented mean ±SE for 5 mice/group. Ketamine was injected alone ( 71.95mg/kg, IP) or with xylazine (14.156mg/kg, IP).

(\*): Significantly change with the control group at P ≤0.05

(a) : Significantly change with the ketamine alone group at P ≤0.05

at dose 71.95 mg/kg i.p. and Xylazine 14.156 mg/kg i.p. causes increased glial cells, vascular congestion ,vasogenic edema, cellular edema, inflammatory cell infiltration meninges and the increase and clustering of glial cells around neurons, Satellitosis (Fig 3,5,7) .

**kidney:** ketoprofen at a dose of 71.95 mg / kg IP. After the fifth and twenty-tenth day of injection showed histological changes in the kidney including: atrophy of the glomerulus , dilatation of Bowman's capsule , vascular congestion , hemorrhage , slight infiltration of inflammatory cells , cellular swelling and epithelial cell necrosis, and vascular congestion fig (9,11,13). While Ketoprofen at dose 71.95 mg/ kg IP together Xylazine 14.156 mg/kg IP. showed cellular swelling ,sever necrosis of the epithelial cells lining the renal tubules , the presence of inflammatory cells, the majority of which are mononuclear (10, 12, 14).

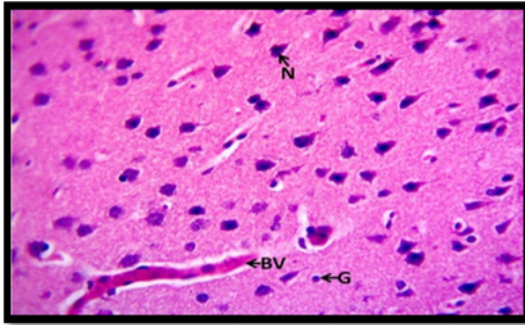
**Liver:** ketoprofen at 71.95 mg / kg IP. after the fifth and twenty-tenth day of injection showed histological changes in the liver including : decentralized venous congestion, necrosis of hepatocytes, enlargement and congestion of sinusoids, slight of inflammatory

cells infiltration, coagulative necrosis , cellular swelling of hepatocytes, focal inflammatory cells infiltration, congestion of the central vein , diffuse coagulative necrosis of hepatocytes , accumulation of polymorphonuclear and mononuclear inflammatory cells around and in the sinusoids , vacuolar degeneration of hepatocytes and Sinusoidal dilatation fig (16,18,20). While Ketoprofen at dose 71.95 mg/kg IP. and Xylazine 14.156 mg/kg IP. causes focal coagulative necrosis of hepatocytes , infiltration of inflammatory cells around them , congestion and expansion of sinusoids , severe coagulable necrosis of hepatocytes ,appearance polymorphonuclear and mononuclear inflammatory cells around blood vessels , vacuolar degeneration of hepatocytes and vascular congestion , focal coagulative necrosis of hepatocytes around the blood vessels , appearance of inflammatory cells around them , cellular swelling of hepatocytes and enlargement of the sinusoids Fig. (17,19,21).

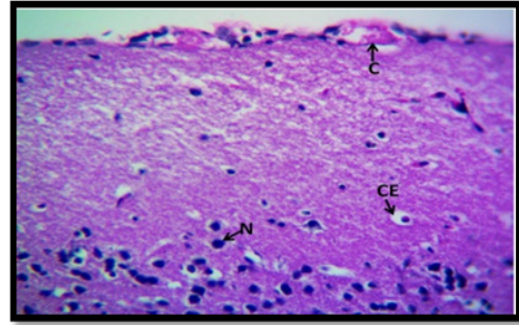
### Discussion

In recent years, several publications on the toxic effects of non-steroidal anti-inflammatory drugs on the liver, brain, and kidney have been published. This research is focused on the toxicity

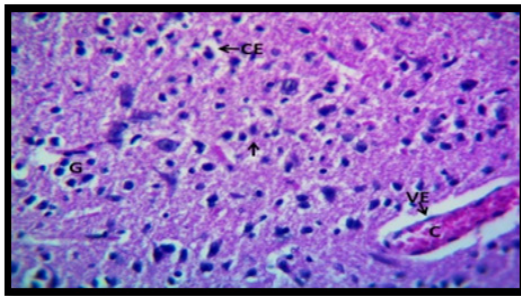




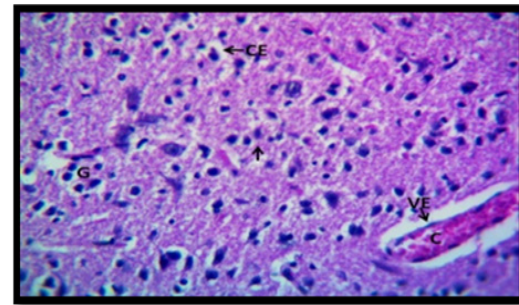
**Fig 1:** Histological section of the brain normal mice showing the cerebral cortex represented by neurons (N), glial cells (G), and blood vessels (BV). H & E, 400X



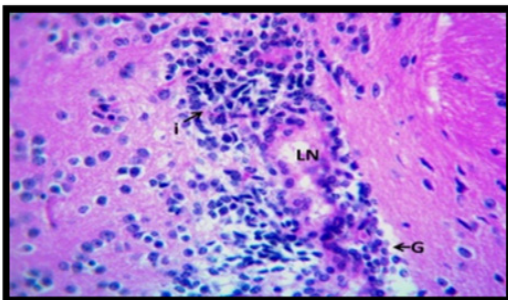
**Fig 2:** Histological section of the brain of mice from the group treated with Ketoprofen (after 5 days), showing the normal structure of neurons (N), congestion of the meningeal blood vessels (C), and vasogenic edema (CE). H & E, 400X



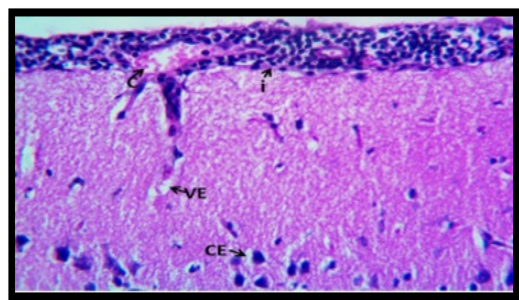
**Fig 3:** Histological section of mice brain from the group treated with ketoprofen and xylazine (after 5 days) showing increased glial cells (G), vascular congestion (C), vasogenic edema (VE), and cellular edema (CE). H & E, X400



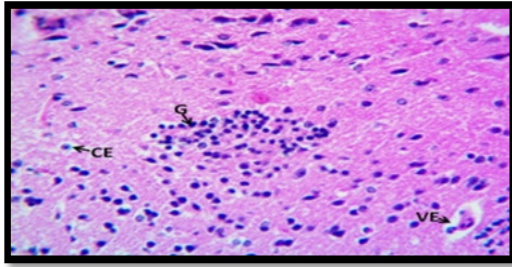
**Fig 4:** Histological section of the brain of mice from the ketoprofen-treated group (after 10 days), showing necrosis of the nervous tissue (LN) surrounded by glial cells (G) and inflammatory cells (i). H & E, 400X



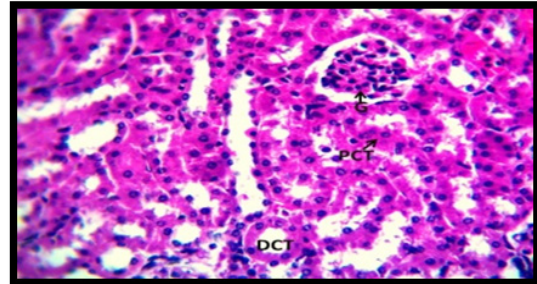
**Fig 5:** Histological section of the brain of mice from the group treated with ketoprofen and xylazine (after 10 days), showing infiltration of inflammatory cells in the meninges (G), vascular congestion (C), vasogenic edema (VE), and cellular edema (CE). H & E, 400X



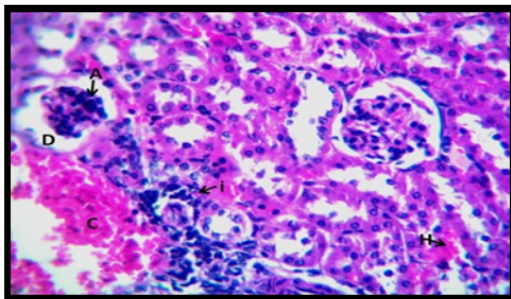
**Fig 6:** Histological section of the brain of mice from the group treated with ketoprofen (after 20 days), showing the presence of angioedema (VE), cytoedema (CE), and increased glial cells (G). H&E,400X



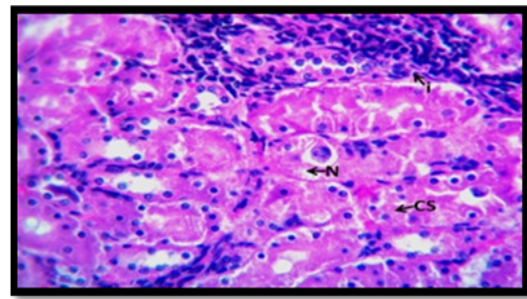
**Fig 7:** Histological section of the brain of mice from the group treated with ketoprofen and xylazine (after 20 days), showing the increase and clustering of glial cells around neurons,



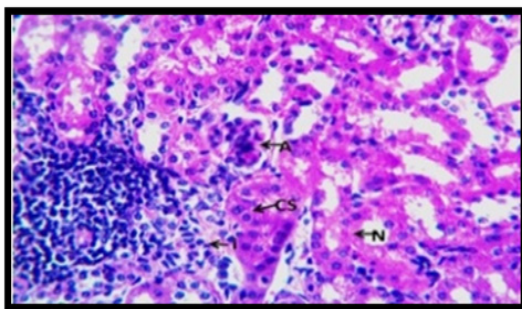
**Fig8:** Histological section of a mice kidney from the control group showing normal histological features represented by normal renal glomeruli (G) surrounded by proximal renal tubules (PCT) and distal renal tubules (DCT). H & E, 400



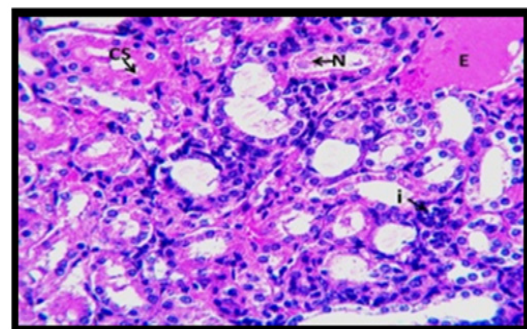
**Fig9:** Histological section of mice kidney from the Ketoprofen-treated group (after 5 days) showing atrophy of the glomerulus (A), dilatation of Bowman's capsule (D), vascular congestion (C), hemorrhage (H), and slight infiltration of inflammatory cells (i). H &E, 400X



**Fig 10:** Histological section of a mouse kidney from the group treated with ketoprofen and xylazine (after 5 days), showing cellular swelling (CS) and necrosis (N) of the epithelial cells lining the renal tubules and infiltration of inflammatory cells (i). H&E, 400X

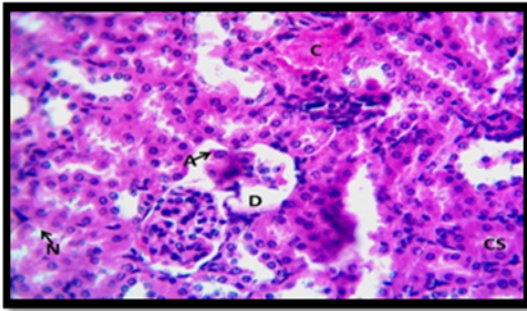


**Fig 11:** Histological section of mice kidney from the group treated with Ketoprofen (after 10 days), showing atrophy of the renal glomerulus (A), cellular swelling (CS), necrosis (N) of the epithelial cells lining the renal tubules, and infiltration of inflammatory cells (i).H&E, 400X

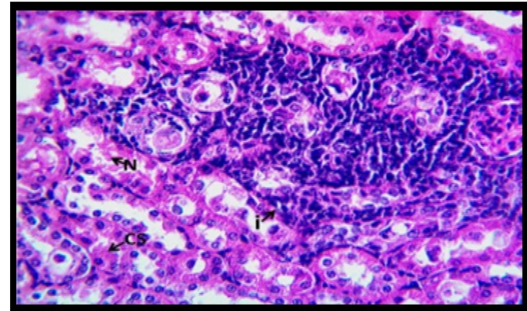


**Fig 12:** Histological section of mice kidney from the group treated with ketoprofen and xylazine (after 10 days), showing cellular swelling (CS), severe necrosis (N) of the epithelial cells lining the renal tubules, edema (E) and infiltration of inflammatory cells (i). H&E, 400X

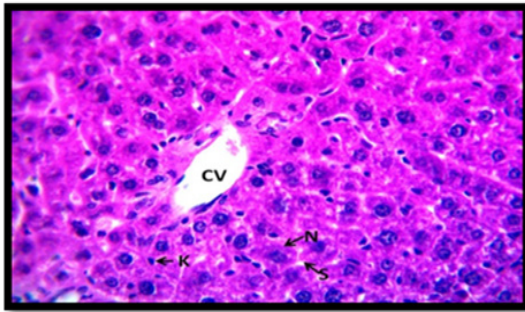




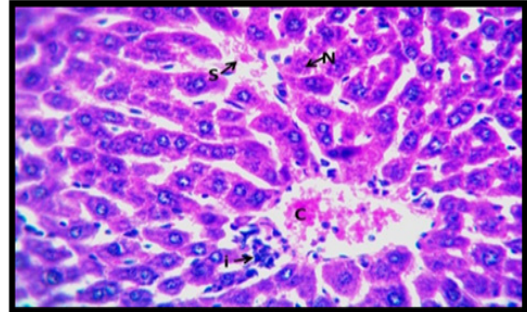
**Figure 13:** Histological section of mice kidney from the ketoprofen-treated group (after 20 days) showing atrophy of the renal glomerulus (A), Bowman's capsule expansion (D), cellular swelling (CS), necrosis (N) of the epithelial cells lining the renal tubules, and vascular congestion (C). H&E,400X



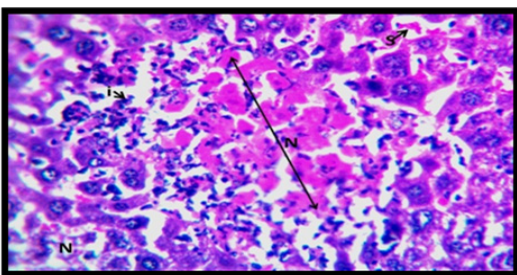
**fig 14:** Histological section of mice kidney from the group treated with Ketoprofen and Xylazine (after 20 days) showing cellular swelling (CS) and severe necrosis (N) of epithelial cells lining the renal tubules and dense focal infiltration of inflammatory cells,



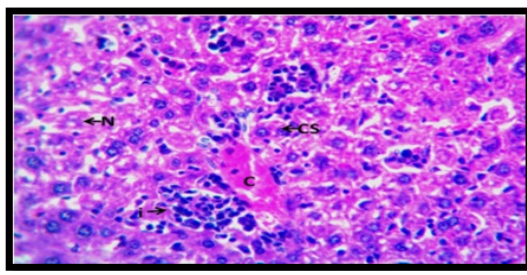
**fig 15:** A histological section of mice liver from the control group showing the normal shape of the central vein (CV) and the arrangement of hepatocytes around it in the form of bands (H), sinusoids (S), and Kupffer cells (K). H&E,400X



**Fig 16:** Histological section of mice liver from the group treated with Ketoprofen (after 5 days), showing decentralized venous congestion (C), necrosis of hepatocytes (N), enlargement and congestion of sinusoids (S), and slight infiltration of inflammatory cells (i).H&E, 400X

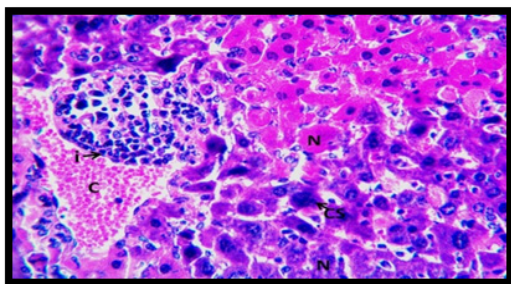


**Fig 17:** Histological section of mice liver from the group treated with ketoprofen and xylazine (after 5 days), showing focal coagulative necrosis of hepatocytes (N), infiltration of inflammatory cells around them (i), and congestion and expansion of sinusoids (S). H&E, 400X

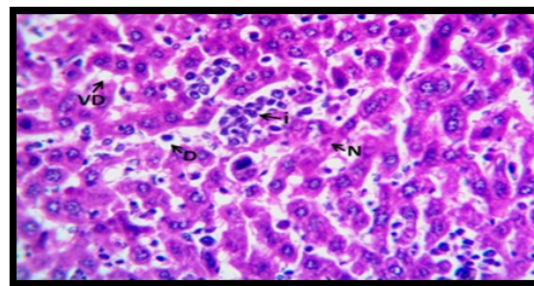


**fig 18:** Histological section of mice liver from the group treated with Ketoprofen (after 10 days), showing coagulative necrosis (N), cellular swelling (CS) of hepatocytes, focal infiltration of inflammatory cells (i), and congestion of the central vein. (C) H&E, 400X

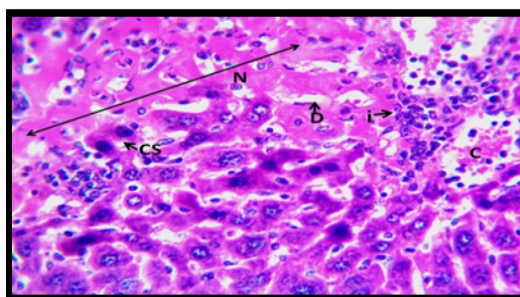




**fig 19:** Histological section of mice liver of the group treated with ketoprofen and xylazine (after 10 days) showing severe coagulative necrosis of hepatocytes (N), infiltration of polymorphonuclear and mononuclear inflammatory cells around and in the sinusoids (i), and vacuolar degeneration of hepatocytes (VD), and vascular congestion (C). H&E, 400X



**fig 20:** Histological section of mice liver of the group treated with Ketoprofen (after 20 days) showing diffuse coagulative necrosis of hepatocytes (N), infiltration of polymorphonuclear and mononuclear inflammatory cells around and in the sinusoids (i), vacuolar degeneration of hepatocytes (VD), and Sinusoidal dilatation (D). H&E, 400X



**fig 21:** Histological section of mice liver of the group treated with ketamine and xylazine (after 20 days) showing focal coagulative necrosis of hepatocytes around the blood vessels (N), infiltration of inflammatory cells around them (i), cellular swelling of hepatocytes (VD) and enlargement of the sinusoids (D). H&E, 400X

of ketoprofen in the present of xylazine in mice. By measuring the  $LD_{50}$  by up and down method, ketoprofen was 359.75 mg/kg, and xylazine was 70.78 mg/kg, while the  $LD_{50}$  of xylazine in chicks was 65.26mg/kg intramuscular [19]. The most prominent symptoms of toxicity that appeared in mice were lethargy, writhing, hair erection, and slow breathing. The change in the  $LD_{50}$  may be due to the variety of the kind of animal and the route of treatment.

#### Neurobehavioral test

The hypoactivity in the open-field test, due to depression in the CNS [20]. Lengthens in the performance of negative geotaxis due to a defect in the vestibular function [21], and the head poking test, which reveals the negative effects on

the brain and its cognitive function, measured in the current study [22]. In addition to the decreased performance on the swimming test brought on by a problem with the muscular system's reaction to neurological system stimulation and the central nervous system's synchronization with the muscles [23]. Ketoprofen or ketoprofen and xylazine causes significant decline in (lines crossed and rearing), head poking, significant elevation in the duration of negative geotaxis performance, these results agreement with other study [26]. The results of neurobehavioral tests indicate the depressants effect of ketoprofen and xylazine on the brain.

Drug-induced liver injury is monitored and diagnosed using blood biomarkers for

general liver injury, such as (ALT) and (AST), an intracellular enzyme that can suggest harm to hepatocytes or biliary cells [27]. ALT and AST are the most sensitive markers, responding quickly to liver cell abnormalities [28,29]. AST is present in both cytosolic and mitochondrial forms in the liver, heart, skeletal muscle, kidney, brain, pancreas, and lung tissue as well as in white and red blood cells. In contrast, ALT is thought to be more specific for liver injury than AST because it is present primarily as a cytosolic protein in the liver and in low concentrations elsewhere [30]. We recorded values of the enzyme indices (AST, ALT,) elevated during ketoprofen injected for 10 days and ketoprofen with xylazine for 5,10 and 20 days in mice. Our results agree with previous study in mice [31]. and Mousa *et al.*[32]. in chicks. Elevated serum levels of these enzymes are a symptom that the hepatocyte membrane's structural and functional integrity has been lost [33].

Ketoprofen or ketoprofen with xylazine injected for 5, 10, and 20 days showed significant elevated in values of uric acid and creatinine concentration comparison with the control group. Similar results were obtained by many authors [34,35,36].

The kidneys' glomeruli typically filter urea and creatinine, two metabolic waste products [37]. Because prostaglandin synthesis is inhibited by NSAIDs, kidney function is known to be altered. As a result, urea, creatinine, and other nitrogen waste products that are typically removed by the kidneys are retained [38-41]. As a result, renal damage may be indicated by serum amounts of urea and creatinine [42,43]. On the other hand, Borges *et al.* found that the levels of urea and creatinine barely changed [44]. The concentration of serum urea and creatinine did not significantly change, according to Muchhara *et al.* [45]. In addition, Ibuprofen treatment to rats did not alter blood concentration of urea and creatinine in low and high doses at 7 days and in low dose at 14 days, but this concentration did increase in high dose at 14 & 28 days of the trial, according to Aprioku *et al.* [46].

#### *Histopathological effects in liver:*

Our results of histological section of the liver in mice treated with ketoprofen after 5, 10 and 20 days indicated inflammatory cells

infiltration, coagulative necrosis and expansion of the sinusoids, and this was proven by the researchers in previous studies [31,47,48]. The liver's histology shows considerable expansion of sinusoidal capillaries and modest perisinusoidal fibrosis, but neither the centrilobular veins nor the portal veins show any discernible alterations. In the presence of leukocyte infiltrates, the hepatic parenchyma responded to this chemical. Additionally, venous shunts and necrosis sites were seen in some parenchymal regions. Hepatocytes in the proximity of porous places have nuclei with apparent chromatic material, and their cytoplasm is essentially nonexistent [31].

Histopathological results of kidneys of the sacrificed mice treated with Ketoprofen or ketoprofen and xylazine for 5,10, and 20 days revealed marked nephropathic lesions in renal tubules and Bowman's capsule expansion, Similar results were recorded by many authors [48,49,50,34]. Some studies clarified how Ketoprofen works and what it does. Like all NSAIDs, ketoprofen works by preventing arachidonic acid metabolism through the cyclooxygenase (COX) pathway [51]. The afferent arterioles of the glomeruli are dilated by prostaglandins, which also maintain the glomerular filtration rate [52]. By inhibiting the COX pathway and counteracting prostaglandin's protective effects, NSAIDs activate the lipooxygenase pathway and increase the production of leukotrienes, which function as mediators of inflammation [53,54]. Furthermore, NSAIDs reduce the kidneys' capacity to automatically control blood flow [55,56].

Histopathological results of the brain of the sacrificed mice treated Ketoprofen or ketoprofen and xylazine for 5,10, and 20 days revealed marked congestion of the meningeal blood vessels, vasogenic edema, cellular edema, necrosis of neuronal cells, increase of inflammatory cells. While Ketoprofen at dose 71.95 mg/kg IP. and Xylazine 14.156 mg/kg IP. causes increased glial cells, vascular congestion, vasogenic edema, cellular edema, infiltration of inflammatory cells in the meninges and the increase and clustering of glial cells around neurons, Satellitosis. Our results were similar to what the researchers reported [19,57,58]. When xylazine and ketamine are used to anesthetize monkeys, the results include rapid neurological damage to the brain and spinal cord

brought on by a number of reasons, including the hypotension brought on by the xylazine [59].

### **Conclusion**

From our results, we concluded that ketoprofen has toxic effect in mice represented by neurobehavioral biochemical and histopathological changes increase by the presence of xylazine. Microscopic damages of the liver, brain, and kidney were more frequent in the mice injected with both ketoprofen and xylazine than in those injected with ketoprofen alone .

### *Conflict of interest*

The author says there are no competing interests.

### *Contribution of the author*

Except for the histopathological reading, which was completed by a pathology expert, all work was completed by the author.

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## تأثير اعطاء الزيلازين على سمية الكيتوبروفين في الفئران

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كان الهدف من دراستنا هو تقييم تأثير الزيلازين على سمية الكيتوبروفين في الفئران ، ثم حددنا تأثير الإعطاء المشترك للزيلازين والكيتوبروفين على السمية السلوكية العصبية ووظائف الكبد والكلية وكذلك التأثيرات المرضية النسيجية بعد الإعطاء اليومي المتكرر لمدة 20 يوماً. كانت الجرعة المميتة (LD<sub>50</sub>) لكيتوبروفين والزيلازين على التوالي (375 ملغم / كغم ، 75 ملغم / كغم) على التوالي. تسبب حقن الكيتوبروفين بمفرده بجرعة (0.126 ملغم / كغم) والكيتوبروفين والزيلازين معاً بجرعة (0.663 ملغم / كغم) في انخفاض في عدد المربعات المقطوعة والوقوف على الاطراف الخلفية بشكل ملحوظ. رافق ذلك زيادة كبيرة في الوقت الذي يستغرقه الفأر للعودة إلى وضعها الطبيعي وانخفاض كبير في عدد مرات ادخال الرأس في الثقب . في اليوم الخامس والعاشر والعشرين من اخذ الكيتوبروفين (71.95 ملغم / كغم) والزيلازين (14.156 ملغم / كغم) كان هناك ارتفاع كبير في تركيز إنزيمات الكبد (AST & ALT) ووظائف الكلى (الكرياتينين و حمض البوليك) ، رافقه تغيرات نسيجية في الكبد والكلية والدماغ. تكشف النتائج التي توصلنا إليها أن الزيلازين تسبب في تقاوم سمية الكيتوبروفين والتي تظهر كتأثير مثبط على الجهاز العصبي المركزي والكبد والكلية ، علاوة على التغيرات النسيجية المرضية في الكبد والكلية والدماغ.

**الكلمات المفتاحية :** الكيتوبروفين ، الزيلازين ، التسمم ، الفئران.