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Research Article

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Histological and Biochemical Evaluation of Acetaminophen Intoxication for Adult Rats: Effects on Liver and Kidney

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ABSTRACT

The toxicity of acetaminophen taking in toxic doses per body weight of 12 adult rats, over a period of 12 days, allowed the biochemical and histological evaluation of the intoxication risks over time. The twelve rats, male and female of 100 grams as average weight, were divided into four groups (of three rats each), were exposed to different increasing doses of acetaminophen 200, 800, 1400 and 2000 mg/kg. The hepatic cytolysis and the congestion of the renal parenchyma were observed in all rats. Biochemically, since the first four days, there was an increase of AST noted with high doses of acetaminophen, and a discrete elevation of creatinine. Tracking -histologically- the toxicity over time shows that renal congestion regresses despite the continuation of toxicity. Contrary, in the liver, cytolysis shows no regression during the time of the experiment. This study allows to highlight some acetaminophen intoxication risks. These risks seem higher than what is commonly admitted.

Keywords: Acetaminophen; Toxicity; Histology; Biochemistry; Liver; Kidney

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INTRODUCTION

Acetaminophen is an analgesic; it is a part of composition of about sixty pharmaceutical specialties and can be in different forms. The maximum human dose depends on the recommendation for each country. In France, the recommendation is: 500 g in 4 hours. Unlike non-steroidal anti-inflammatory drugs, acetaminophen does not affect platelet aggregation. It has a little contraindication, can be prescribed for any age without serious side effects when used at the recommended dose. However, it is an analgesic of level 1 (WHO), its power remains moderate. Despite its favorable risk profile, acetaminophen is one of the main causes of drug intoxication and poisoning deaths. In the US, the acetaminophen intoxication is the leading cause of hepatic failure or insufficiency [1]. In Switzerland, the toxicological information center inventoried, in 2012, more than 1,200 calls related to acute acetaminophen intoxication. This number is constantly increasing since 1995 [2], two thirds of the calls were from adults. 13% of patients had moderate to severe intoxication. The aim of our work is to search by an experimental scientific work, the effects of the acetaminophen overdose on laboratory rats. This research tends to demonstrate that high doses of this drug, can have side effects such some significant cell damages.

MATERIALS AND METHODS

Our study has focused on assessing the acetaminophen toxic impact, by using toxic doses in 12 adult rats during 12 days. For this, the histological experiment part was done at the laboratory of histology, embryology and cytogenetic in Biology department of the Faculty of Medicine and Pharmacy at the Mohammed V University in Rabat. The determination of hepatic and renal assessments was carried out in collaboration with a medical analysis laboratory.

Experimental Animals

The experiment was conducted on a single species. These are the male and female rats of Wistar albinos, aged –at least- 90 days and weighing between 90 and 100 grams. The rats were bred in the same conditions of ventilation, sufficient brightness and ambient temperature. They were regularly fed on a standard diet composition consisting of maize, wheat, and sunflower nuggets. They had free access to food and water.

Experimental Protocol

12 rats, with average weight of 100 grams. They were divided into four groups of three rats each, under the same experimental conditions; exposed to different doses of acetaminophen: non-toxic doses (200 mg/kg), doses may be toxic (800 mg/kg), moderately toxic doses (1400 mg/kg) and highly toxic doses (2000 mg/kg). Each group corresponds to a dose. Two other supplementary rats were categorized as controls and received distilled water to be compared with experiment rats. We calculated the acetaminophen doses to be administered to rats in the following manner (Table 1):

Table 1: Administrated doses in mg/kg for rats of about 100 g

Degree of toxicity	Conventional doses in mg/kg	Administered doses in mg/kg body weight
Maximum toxic dose	2000	200
Moderately toxic dose	1400	140
Dose likely to be toxic	800	80
Non-toxic dose	200	20

Experimental Steps (Figure 1)

The first day: administration by gavage of medication doses, corresponding to each group.
The 4th day: blood sampling, then, sacrifice one rat in each group.
The 8th and 12th day from the gavage date: sacrifice one rat in each group.

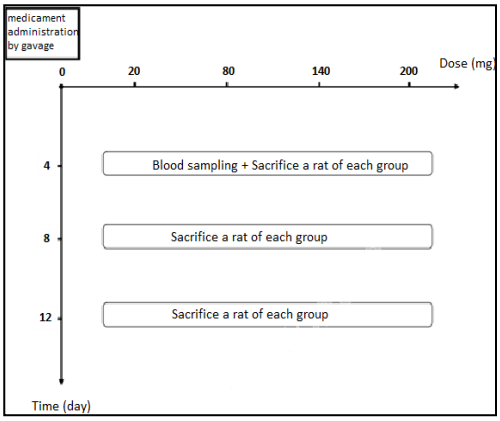

Figure 1: The followed experimental protocol

Methods

Preparations and administration of the product:

In order to check the toxic effect, the rats of different groups received acetaminophen by gavage once for each group by increasing doses as mentioned above, using a probe attached to a syringe.
For this, 500 mg acetaminophen pills were milled into a fine powder. Each dose was measured by a precision balance and then placed in 4 ml of distilled water. The gavage was carried out using a gastroesophageal probe.

Experimental part:

By respecting the chronology of our protocol:
-At the 4th day, blood test was done for all groups. These samples were made in tubes without anticoagulant and centrifuged at 4000 rpm/min for 10 minutes. The collected serums stored at -20°C, were used to perform liver function tests by transaminases dosage (ALT and AST) and renal function tests by urea and creatinine dosage.
Then we proceeded to the sacrifice of one rat from each group for histological examination of target organs (liver, kidney).
-For The 8th and 12th days, for technical reasons out of our control, we were not able to perform dosage of transaminases and renal function tests, so we settle for the only histological study.
For histological study:
Organs were preserved in 10% formol for 48 hours.
After inclusion to paraffin of the organ fragments, sections of 4 microns with a microtome were performed and stained with hematoxylin-eosin, and then fixed between slide and cover slip before being observed by using a microscope equipped with a camera.

RESULTS AND DISCUSSION

Acetaminophen is a commonly used drug and available in pharmacies. The related overdose cases are common, more than 100,000 per year in the United States, a hundred in Switzerland, with sometimes serious consequences.
Acetaminophen -properly used- at therapeutic doses is safe, by against, misused or at high doses, can cause -sometimes- fulminant and irreversible lesions [3,4], particularly in the livers of alcoholics [5].
In this work, we are interested in studying the effects of acetaminophen intoxication, by dosing some serum parameters and histological examination of target organs.
The authors agree that acetaminophen overdoses in humans, are common and they occur in many different ways by touching particularly the liver and the kidney [6], especially in patients with previously impaired liver function.
In animals, acetaminophen is toxic for cats and dogs … For the laboratory rats, non-toxic doses are considered around 50 - 150 mg/kg, 1500 mg/kg is moderately toxic dose, and the highly toxic doses can reach up to 2000 mg/kg [7]: this is the model we have chosen for our study.

Biochemical Parameters

Transaminases dosage:

Hepatic function of animals from the 5 groups (control, 20 mg, 80 mg, 140 mg and 200 mg) was explored by serum liver enzymes: ALT and AST in the 4th day of the study. The results are shown in the Table 2 and illustrated in the Figure 2.

Table 2: Assessment of biochemical parameters (ALT - ALT) based on doses received by rats

	Doses (mg/kg BW)				
Transaminases	CG (control group) (0)	G1 (20)	G2 (80)	G3 (140)	G4 (200)
AST (IU/L)	144, 07	173, 41	35, 82	567, 82	577, 47
ALT (IU/L)	77, 15	101, 57	210, 24	129, 75	121, 45

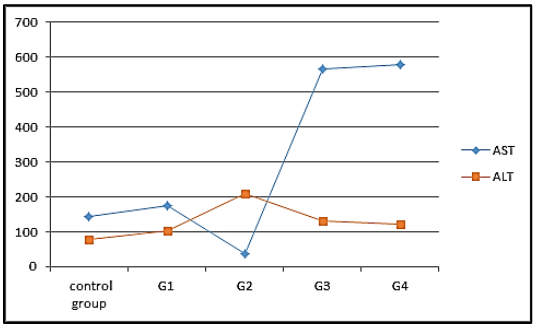

Figure 2: Assessment of biochemical parameters (ALT - ALT) based on doses received by rats

The analysis of the figure shown above confirms that the 1st, 3rd and 4th group have an increased transaminases content (AST, ALT) compared to the control group. The results of the 2nd group (G2) appear non compatible. It seems that this is probably due to a wrong manipulation at different times of the analysis, therefore we eliminated the result of the G2 in our study. Interpretation of liver enzyme results shows, concerning AST, for the 3rd and 4th group (high doses) we observe a very significant increase almost fivefold compared to normal, while for ALT levels

share an almost identical average normality. We share virtually the same results as those published by Durand F [6], for which the activity of AST is most important in overdose by acetaminophen case. Physiologically, ALT is expressed mainly in the liver, while the AST are more active in the muscle. Thus in liver disease, elevated ALT is greater than the elevation of AST, while in muscle diseases, elevation is reversed [8]. If this assertion is true, we are allowed to assume concomitant muscle damage to liver damage. Since the rats showed no locomotive disorders, has not prompted us to make a histological study of the muscle. Therefore, the profiles of AST and ALT should be explored as well in humans as in rats to precise the muscle and the liver alterations in acetaminophen intoxication.

Urea and creatinine dosage:

Renal function of the 5 groups animals (control, 20 mg, 80 mg, 140 mg and 200 mg) was investigated by urea and creatinine dosage on the 4th day of the study. The results are shown in the Table 3 and illustrated in Figure 3.

Kidney failure (marked by an increase in creatinine serum) is common in patients who have ingested large doses of acetaminophen, but it is not constant [9,10].

Table 3: Assessment of biochemical parameters (urea - creatinine) based on the doses received by rats

	Doses (mg/kg BW)				
Parameters	Control group	G1	G2	G3	G4
Urea (g/l)	0.34	0.2	0.22	0.27	0.16
Creatinine (mg /l)	5.7	5.21	6.83	6.38	6.61

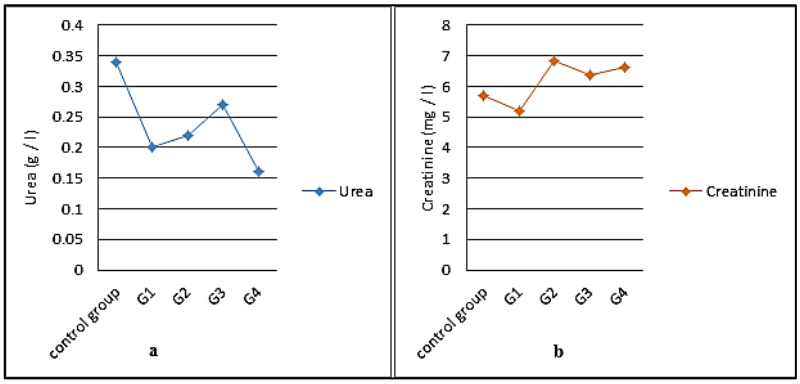

Figure 3: Evaluation of biochemical parameters (urea - creatinine) based on the doses received by rats

Regarding our results, we can see from the table and the figure that the results of the exploration of these parameters remain practically in normality, apart an insignificant slight increase in the rate of creatinine for high doses (Figure 3b). McClain et al. [11] have shown that there is an increased creatinine, from our side we can therefore affirmed that there is an increase in the same parameter, which is more transient as we will show in our histological study of the kidney.

Histopathology

Liver histopathology:

In humans, the liver biopsy shows lesions involving predominantly centrilobular hepatocyte necrosis, more or less extensive, with rare inflammatory infiltrates. When the necrosis has already been resorbed, a collapse of hepatocyte spans is observed reflecting the disappearance of hepatocytes [6,12]. In our work we found that: At low doses (20 mg) no abnormality is observed and for the 2nd group (received a 80 mg dose), even in the early days there is a congestion of the liver parenchyma without any other anomaly; that congestion tends to decline gradually up the 12th day (Figure 4).

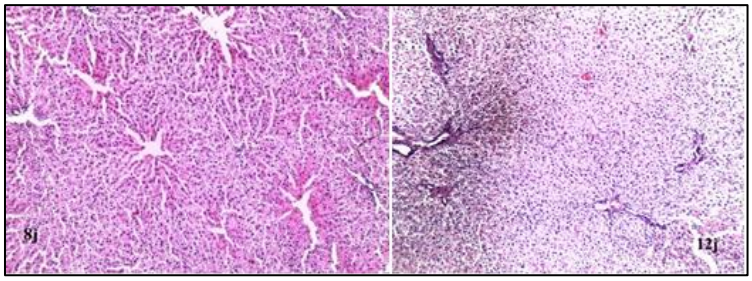

Figure 4: Histological sections of liver tissue (8th and 12th day)

At high doses (140, 200 mg), congestion is more important, the whole architecture is well respected. On agreement with Abdel-Daim et al. [13], the hepatic cells show cytolysis images firstly on the periphery of the lobule respecting the centrilobular area, and then they tend to spread across the major part of the Remack bay. Some authors report that the lesions affecting the outset centrilobular vein without specifying the status of hepatocytes [14,15]. The originality of our study is to have been able to follow the progression of lesions over time, which shows that the lesions are firstly peripheral then centrilobular (Figure 5).

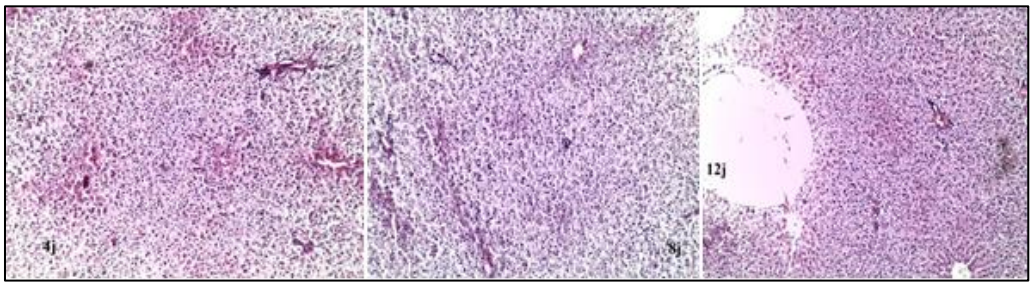

Figure 5: Histological sections of liver tissue of the 4th group (200 mg) over time (4, 8, 12th day)

Our results are therefore agrees exactly with literature data [16,17]. On the theoretical level, the pathophysiology of injuries is explained by the metabolism of acetaminophen that produces the toxic molecule, *N-acetyl* benzoquinone imine (NAPQI) via cytochrome P450. This metabolite can cause death of liver cells. It is removed, in the liver, by reaction with glutathione which captures radicals. Indeed, when the acetaminophen dose is too large, the NAPQI is produced in large quantities, glutathione reserves are depleted and the liver can no longer remove it; then, it will undergo more or less significant damage depending on the amount of absorbed acetaminophen [18].

This overdose can cause hepatitis with severe liver damage (hepatic cytolysis), leading to necrosis in extreme cases. The consequences of an overdose are severe, sometimes fatal. The liver damage is irreversible and a liver transplant becomes necessary when the damage is very important. The NAPQI creates adducts attached to hepatic proteins, membrane lipid degradation and disruption of calcium homeostasis, causing necrosis and cytolytic hepatitis [19]. The hepatic cytolysis observed in our study, is corroborated by the increase in AST to high doses of acetaminophen, as we have indicated above (Figure 2). The doses that we administered to rats are very high related to its weight, reported in humans will be overly toxic; it can cause severe cytolysis and extreme death of the individual, as is the case of rats in which we administered these doses.

Histopathology of kidney:

In the opinion of most authors [20,21] the kidney is a target organ for acetaminophen intoxication. In our study respecting the experimental protocol, we get the following results: At low doses (20, 80 mg), the study of histological kidney on day 4 (Figure 6), shows a relatively well-preserved architecture, The glomeruli are well designed with the conservation of the Bowman's capsule, contrary to what was reported by Adeneye AA et al. [22]. However, in places, dissociation of structures is observed; the tubes seem–in this level- hydropic and dissociated.

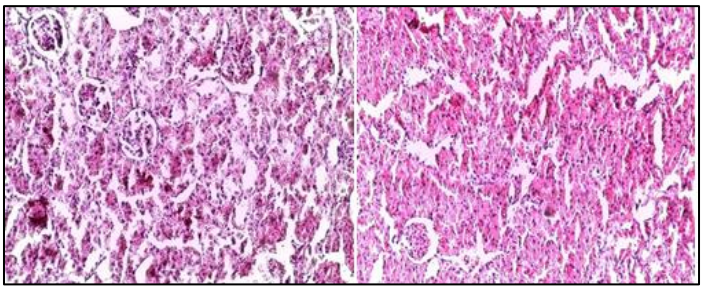

Figure 6: Histological sections of renal tissue of the 1st and 2nd group (20, 80 mg) on 4th day. HE staining / magnification (X20)

Beyond the 4th day, the observed injuries regress significantly (Figure 7).

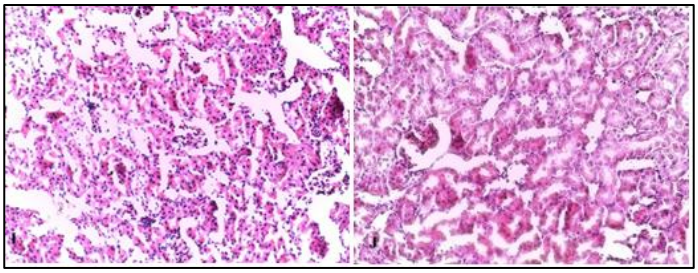

Figure 7: Histological sections of renal tissue of the 1st and 2nd group (20, 80 mg) the 12th day. HE staining / magnification (X20)

At higher doses (140, 200 mg) and in the beginning of intoxication (4th day) (Figure 8), we find that the lesions are identical to those observed at low doses, but they are much more intense with significant retraction of some glomeruli. In addition, we observed the presence of probable pseudo giant cells within the parenchyma probably related to a foreign body reaction. We report as other authors the presence of non-specific inflammatory phenomena that accompany acetaminophen intoxication [23,24].

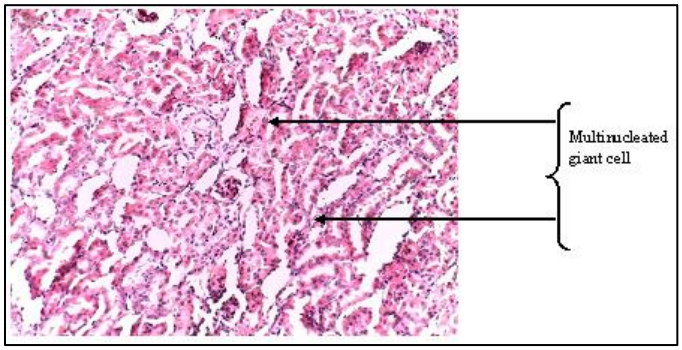

Figure 8: Histological section of the 4th group of kidney tissue (200 mg) in the 4th day. HE staining / magnification (X20)

It should be noted that all these lesions tend to fade gradually until almost totally disappear the 12th day (Figure 9).

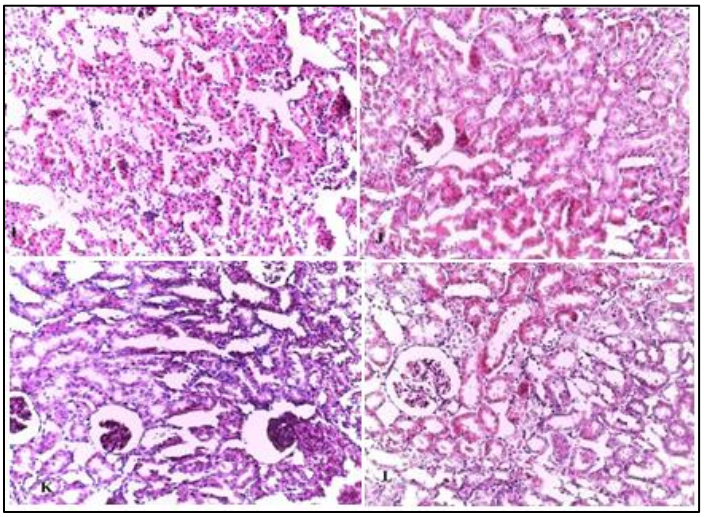

Figure 9: Histological sections of the renal tissue of the 4th groups on the 12th day. HE Staining / Magnification (X20)

Because the metabolism of acetaminophen is mainly hepatic, all writers are almost unanimous that liver damage is primordial. Only 4-5% of the drug is excreted unchanged in the urine, which may explain the correction of disorders in the kidney in the presence of acetaminophen intoxication. In our case, the reduction of renal pathological images after congestive steps and alterations in kidney structure seems to corroborate the literature data. At present, there is insufficient evidence to conclude that the regular use of acetaminophen was associated with an increased risk of chronic renal failure [19], which is not the case of a very serious liver damage.

CONCLUSION

Acetaminophen is widely used for its analgesic and antipyretic properties. The risks associated with its use can sometimes be dramatic especially in the liver. Self-medication is common practice; the risks are potentially serious, in the current context.

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