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Long-term toxicological effects of paracetamol in rats

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Abstract

The analgesic and antipyretic properties of paracetamol were first described in 1893, then it has been widely available as a non-prescription drug, with a therapeutic profile that reflects widespread safety and efficacy as well as paracetamol became the most widely used analgesic and antipyretic in children. It is the most frequently used over-the counter medicine in young children and is nearly universally used in infants. The drug is used by millions of children every day. The study was designed to study the toxicological effect of therapeutic dose of paracetamol after oral administration for three months in laboratory rats (*Rattus norvegicus*) on the heart, kidney and liver. Results showed oral administration of the paracetamol for three months in laboratory rats showed that this drug has a severe damaging effect on most of the vital organs in the body like kidney, liver and heart.

Keywords: Toxicological effect; Acetaminophen; Rat.

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تأثيرات الباراسيتامول السمية لمدة طويلة صالح كاظم مجيد، مخلد عبد الكريم رمضان و وسام منذر

كلية الطب البيطري، جامعة البصرة البصرة العراق

الخلاصة

ان اول وصف للخواص المهدئة و الخافضة للحرارة تم وصفها في عام ١٨٩٣ بعده ذلك تم استخدامه كعلاج بشكل واسع نظرا لانه اظهر منى واسع من فة السمية. كذلك استخدم العقول بشكل واسع كمادة مهدئة و خافضة للحرارة في الاطفال. كذلك يستخدم كعلاج بين اليافعين و حديثي الولادة. وكذلك يستخدم من قبل ملايين الاطفال في كل يوم. صممت هذه الدراسة لمعرفة التأثير السمي للجرعة العلاجية لعقار الباراسيتامول لمعطة عن طريق الفم و لفترة طويلة على بعض الاعضاء مثل القلب و الكبد و الكلية في الجرذان المختبرية. اظهرت النتائج ان التجريب القموي لعقار الباراسيتامول لمدة ثلاثة اشهر تأثيرات ضارة على كثير من الاعضاء المهمة في الجسم مثل لقلب و الكلية و الكبد.

Introduction

Paracetamol (known as acetaminophen in the United States) is one of the most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) (1). It is a rapid, reversible, noncompetitive inhibitor of cyclooxygenase activity and thus products of the arachidonic acid cascade. acetaminophen has been available since the 1950s as an over-the-counter product for pain and fever relief. Acetaminophen has long been recognized as potentially lethal because of dose-related hepatic, and often renal, injury (1,2). Although its metabolism is quite well

understood, the mechanism of acetaminophen toxicity remains somewhat a mystery with recent evidence suggesting that multiple cytotoxic pathways are involved (3). Historically, alcohol use was claimed as an important risk factor, with the accidental nature of acetaminophen toxicity in alcoholics being termed a 'therapeutic misadventure' (3,4). Metabolic synergy between alcohol and acetaminophen appears to enhance the toxic reaction but how important this is clinically remains a matter of debate (5,6). Low dose ingestions having harmful consequences appear to be more likely in association with alcohol use (7). Prior to the 1990s, only a few cases of acetaminophen

poisoning had been reported; acetaminophen toxicity was not listed at all in transplant series until two reports in the late 1990's. The Acute Liver Failure Study Group has compiled data on more than 500 acetaminophen-related ALF cases showing that the number of cases has increased considerably since 1998 (8). Suicidal overdose of acetaminophen is the most frequent form of liver injury in the United Kingdom (7). Most people who attempt suicide admit to the misdeed and seek help early.

Most people are only at risk for liver toxicity if they take more than the normal recommended amount of acetaminophen. Most cases of liver damage occur in people who have taken at least 10-15 grams—more than twice the recommended dose. Many of the emergency room visits and deaths linked to acetaminophen poisoning are due to accidental or intentional overdoses. But some people are more susceptible to acetaminophen toxicity and can experience liver damage even at the recommended dose. A study by the U.S. Food and Drug Administration (FDA) showed that about 20% of people with acetaminophen-related liver toxicity had taken less than the recommended daily amount. For other people, a dangerous dose is not much higher than the recommended dose—that is, the “window” between a therapeutic dose and a toxic dose is smaller for acetaminophen than it is for many other drugs. Some experts also believe that taking acetaminophen for several days in a row may cause a dangerous build-up of the drug in the body (9).

Paracetamol also has direct actions on the kidney (10). demonstrated that in the isolated perfused rat kidney, administration of paracetamol resulted in a decrease in GFR and PGE2. Similarly, in normal human volunteers treated with paracetamol for 3 days, a reduction in urinary PGE2 and sodium excretion was observed. In addition, paracetamol induced a delay in the onset of diuresis after an acute water load (4). Paracetamol also exerts acute and chronic nephrotoxic effects. Acute ingestion of large doses (10–15 g) is characterized by necrosis and damage to the proximal tubule. However, it is recognized from both clinical and experimental studies that much lower doses (500–1000 mg) can produce renal damage, especially in patients with hepatic disease or those taking enzyme inducer drugs (carbamazepine and phenytoin) or in the malnourished (2). Chronic ingestion of paracetamol results in analgesic nephropathy. This is defined as habitual ingestion of an analgesic, which after an insidious onset, leads to renal papillary necrosis and chronic interstitial nephritis with progressive renal failure (6). Epidemiological studies show that longterm regular consumption of paracetamol increases the relative risk of chronic renal disease to 3.2 (3), whereas the odds ratio for end stage renal disease was 2.1 for the heaviest annual intake of paracetamol and 2.4 for cumulative lifetime intake of more than 5000 tablets containing paracetamol (7). In the present

study we report the long-term (90 days) toxicological effects of paracetamol in rats.

Materials and methods

Twenty four *Rattus norvegicus* rats were selected for this experiment and housed in normal photoperiod regime in the laboratory for two weeks to give a chance for acclimatization and for anti-bacterial and anti-parasitic drug administration. Animals were divided into four groups, control group, low dose treated group, intermediate dose treated group and high dose treated group, each group consist of six animals. Animals of the control group were given normal physiological saline, the group of low dose, intermediate dose and high dose treated with acetaminophen orally as 250 mg/kg, 500 mg/kg, and 1000 mg/kg respectively daily for three months. The animals of all groups were killed at the end of the experiment and the organs (liver, kidney and heart) fixed in 10% buffered formalin and sent to laboratory for sectioning at 5 µm and stained with hematoxylin and eosin.

Results

The liver, kidney and heart of the control group were showed normal histological structure (Fig 1-3).

In the group of low dose the liver showed congestion, minimal fibrosis in the periportal area and degenerative changes in the hepatocytes with severe infiltration of inflammatory cells in the subcapsular region (Fig 4). The kidney showed degeneration in the renal tubular epithelium in the cortical region (Fig 5), while in the heart there were moderate vacuolation in the myocardial muscle cells (Fig 6).

In the group of intermediate dose the liver showed more massive fibrosis with severe fatty degeneration of the which indicated by presence of clear vacuoles in the cytoplasm of the hepatocytes (Fig 7), the kidney showed glomerular atrophy with degenerative changes in the renal tubular epithelium (Fig 8), in the heart there is marked vacuolation in the myocardial muscle cells (Fig 9).

In the group of high dose the liver revealed vacuolation of hepatocytes with congestion of the central vein and marked fibrosis in the periportal area with hepatocellular necrosis in the central area and the subcapsular region (Fig 10,11), in the kidney there were degeneration of medullary renal tubular epithelium as well as there were hemorrhage in the glomeruli with glomeriolar atrophy (Fig 12,13), in the heart there were severe vacuolation in the myocardial muscle fibers as well as the severe atrophy and fibrosis specially in the pericardial region (Fig 14,15).

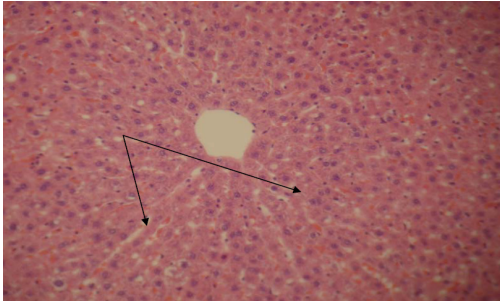


Fig 1: Normal microscopic structure of the liver which shows normal hepatocytes in centrilobular are. H&E 125X.

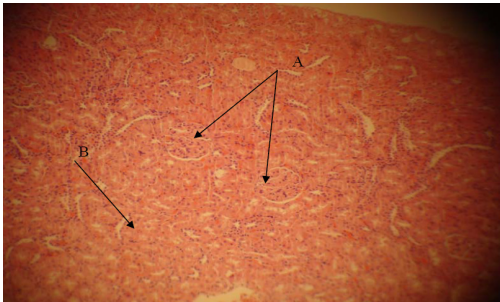


Fig 2: Normal microscopic appearance of the kidney reveale A) the normal glomeruli B) Normal renal tubules in the cortical area.H&E 125X.

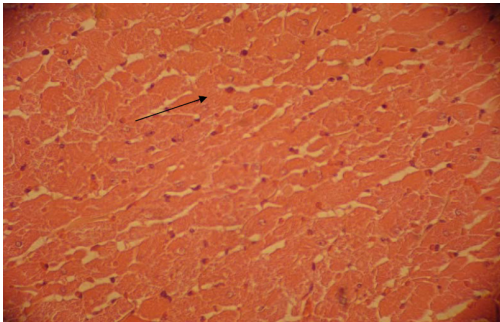


Fig 3: Normal microscopic appearance of the myocardium H&E 125X.

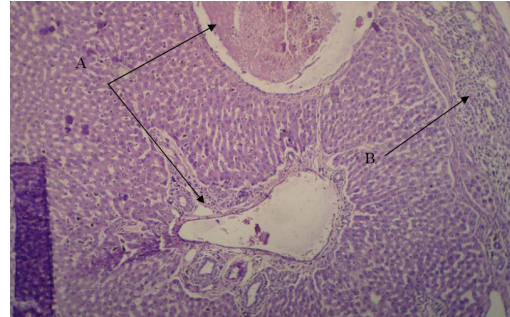


Fig 4: Microscopic appearance of the liver in the group of low dose shows A) conjection with minimal fibrosis in the periportal area B) infiltration of inflammatory cells in subcapsular area H&E 125X.

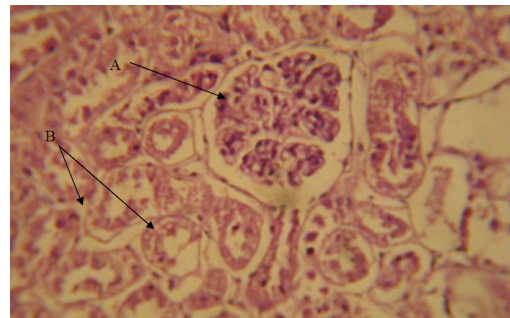


Fig 5: Kidney of low dose group reveal A) slightly atrophied glomerulus B) degeneration of renal tubular epithelium H&E 125X.

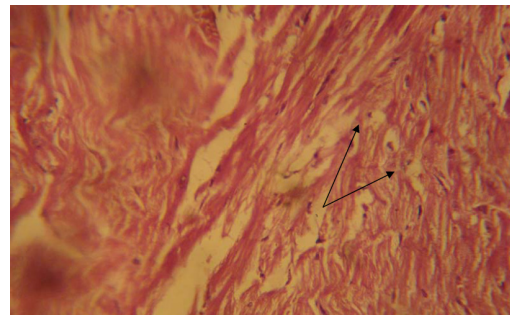


Fig 6: Myocardial muscle of the low dose treated group shows minimal vacoulation of the myocardial muscle cells. H&E 125X.

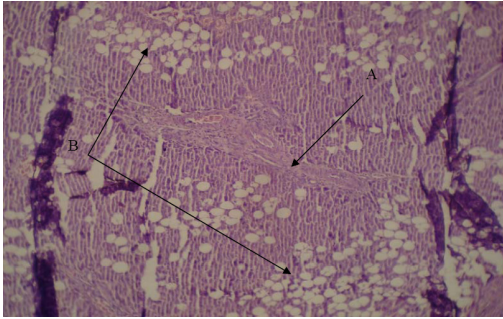


Fig 7: Liver of inter mediate dose treated group shows A) moderate fibrosis in the periportal area B) massive vacuolation of the hepatocytes H&E 125X.

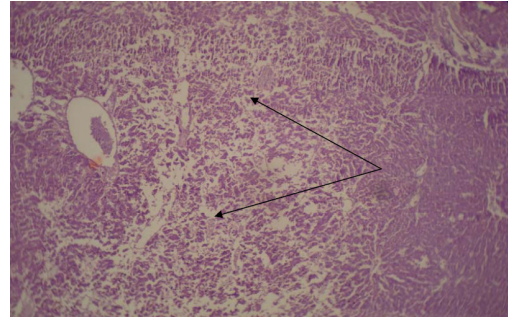


Fig 10: Liver of high dose treated group reveal necrotic change of the hepatocytes in the centrilobular area H&E 125X.

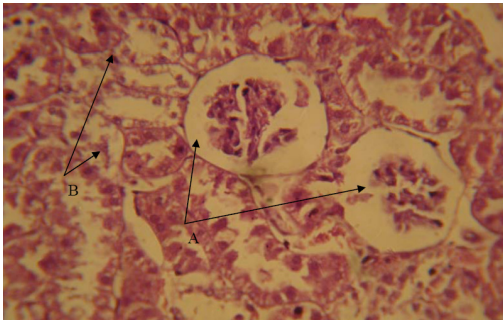


Fig 8: Kidney of intermediate treated group shows A) marked atrophied glomeruli B) degeneration of the renal tubular epithelium H&E 125X.

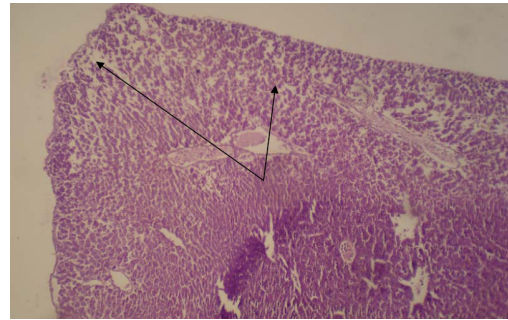


Fig 11: Liver of high dose treated group reveal necrotic change of the hepatocytes in subcapsular area H&E 125X.

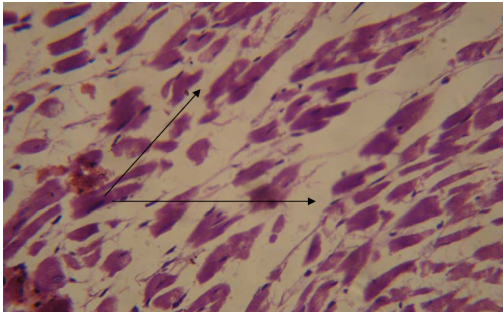


Fig 9: Heart of intermediate treated group shows sever atrophied myocardial muscle cells H&E 125X.

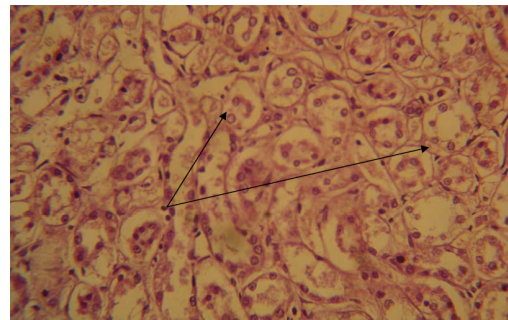


Fig 12: Medullary region of the kidney reveal massive degenerative change in the renal tubular epithelium H&E 125X.

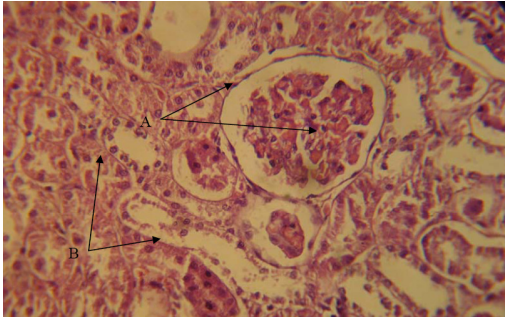


Fig 13: Kidney of the high dose treated group reveal A) glomerular atrophy and hemorrhage B) degeneration of renal tubular epithelium H&E 125X.

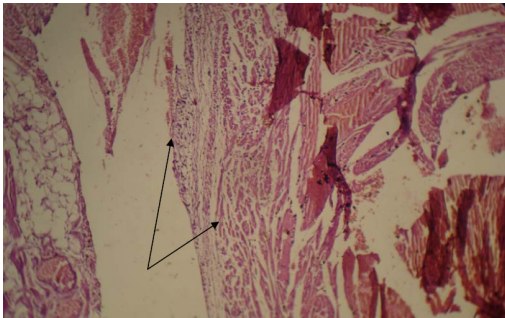


Fig 14: Heart of high dose treated group reveal atrophy of the myocardial muscle cells with fibrosis in endocardial region H&E 125X.

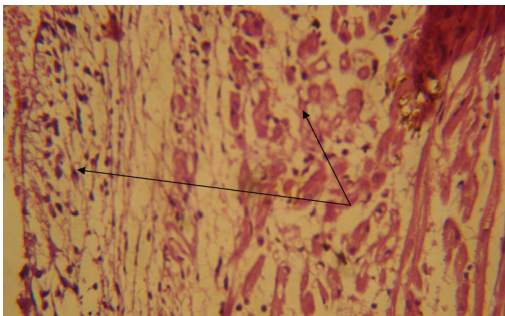


Fig 15: Heart of high dose treated group reveal atrophy of the myocardial muscle cells with fibrosis in endocardial region H&E 125X.

Discussion

Paracetamol is one of the most common antipyretic and analgesic used all over the world. It is easily accessible over the counter and thus intentional paracetamol overdose is common. Ingestion of more than 12.5 g or 25 tablets is associated with acute liver dysfunction.

The renal tubular changes which has been found in this research was agreed with results of other researches which found that The paracetamol induced renal damage results from a mechanism similar to that which is responsible for hepatotoxicity (8,11). Renal failure secondary to acute tubular necrosis occurs in 25% of patients with severe hepatic damage and this result agreed with the result of this research in which most of the animals showed damages in both kidneys and livers and in a few without evidence of serious disturbance of liver function.

The pathophysiological mechanism of paracetamol poisoning is well known. Normally the acetaminophen is mainly metabolised to non-toxic metabolites that are normally excreted. However, in overdose situation, the non-toxic metabolic pathways are saturated and more paracetamol will go through metabolism by the cytochrome P450 pathway, resulting in the accumulation of the toxic metabolite N-acetyl-pbenzoquinoneimine (NAPQI). The NAPQI is detoxified by glutathione until the store is depleted. The NAPQI will then bind to cellular proteins causing damage and cell death (12).

Paracetamol is rapidly absorbed from the small intestine and has a high bioavailability of around 80% after first-pass metabolism. The pathophysiology of paracetamol poisoning is closely related to its metabolism. Paracetamol is predominantly metabolised in the liver by conjugation with sulphate (around 30%) and glucuronide (60%). A smaller amount is eliminated unchanged in urine. However, approximately 5-10% of paracetamol is metabolised to N-acetyl-P-benzoquinoneimine (NAPQI), a toxic metabolite via CYP450-dependent pathways. NAPQI is then detoxified by glutathione and is eliminated in urine or bile. At toxic levels, sulphate and glucuronide conjugation can be saturated. This leads to glutathione depletion as more paracetamol is metabolised through the CYP450 pathways and NAPQI may accumulate. NAPQI that is not detoxified then reacts with sulphhydryl groups on hepatocytes and causes hepatocellular necrosis and this agreed with the result of the present study. In infants and young children, the dominant pathway of metabolism appears to be sulphate conjugation while glucuronide conjugation matures slowly. Children are also thought to have a more active oxidative pathway, resulting in an increased rate of glutathione production, thereby conferring a protective effect from hepatotoxicity in young children. The greater capacity for sulphation in young children alongside an increased incidence of vomiting may also explain why young children

appear less susceptible to hepatotoxicity compared to adults. Despite these apparent protective mechanisms paracetamol may accumulate significantly in children after repeated therapeutic doses. Although no children developed hepatotoxicity after repeated doses of between 66e81 mg/kg/day, increases in AUC of 14e33% were seen. Significant accumulation may explain why children who had ingested multiple supratherapeutic overdoses appear to have worse outcomes (13).

Most of the cardiac muscle change result not from direct effect of paracetamol but could result from the damage of the kidney which cause fluid retention then hypertension and the cardiac damage occur as a consequence for increase blood pressure but we did not find any research to support this result.

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