

# A nutrient mixture prevents acetaminophen hepatic and renal toxicity in ICR mice

MW Roomi, T Kalinovsky, V Ivanov, M Rath and A Niedzwiecki

*Dr Rath Research Institute, Santa Clara, California, USA*

Acetaminophen (APAP) overdose is often fatal, leading to fulminant hepatic and renal tubular necrosis in humans and animals. We studied the effect of a nutrient mixture (NM) containing, among other nutrients, lysine, proline, ascorbic acid, *N*-acetyl cysteine, and green tea extract, which has previously been demonstrated to exhibit a broad spectrum of therapeutic properties on APAP-induced hepatic and renal damage in ICR (Imprinting Control Region) mice. Seven-week-old male ICR mice were divided into four groups (A–D) of five animals each. Groups A and C mice were fed a regular diet for 2 weeks, while groups B and D mice were supplemented with 0.5% NM (w/w) during that period. Groups A and B received saline i.p., while groups C and D received APAP (600 mg/kg) i.p. All animals were killed 24 h after APAP administration. Serum was collected to assess the liver

and kidney functions, and the livers and kidneys were excised for histology. Mean serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, BUN (Blood Urea Nitrogen), creatinine, and BUN/creatinine ratios were comparable in groups A and B, increased markedly in group C and significantly lower in group D compared with group C. APAP caused significant centrilobular necrosis and glomerular damage in unsupplemented animals, while NM prevented these alterations. The results indicate that NM has potential to protect against APAP-induced liver and kidney damage.

**Key words:** acetaminophen; APAP; hepatic; nutrients; renal; toxicity

## Introduction

Acetaminophen, also known as paracetamol and *N*-acetyl-*p*-aminophenol (APAP), is the most widely used over-the-counter analgesic and antipyretic agents in the USA and also the most common drug associated with accidental and intentional poisoning.<sup>1</sup> Acute APAP toxicity can manifest as various degrees of liver toxicity, including acute liver failure. Acetaminophen toxicity is one of the leading causes of liver failure in the USA accounting for more than 56,000 ER visits and 100 deaths per year.<sup>2</sup>

The toxic dose of APAP with a single acute ingestion is 150 mg/kg or ~7 g in adults. It is rapidly absorbed from the stomach and small intestine and metabolized by conjugation in the liver to nontoxic agents, which are eliminated by urine. In acute overdose or prolonged use of the drug, the conjuga-

tive pathways are saturated and excess APAP is oxidatively metabolized in the liver via cytochrome P450 system to a toxic metabolite *N*-acetyl-*p*-benzoquinone-imine (NAPQI). When excess NAPQI is formed and/or glutathione stores are low, NAPQI binds to vital proteins (as an APAP-cysteine adduct and can also oxidize protein thiol groups to disulfides, forming intra- and interprotein crosslinks) and the lipid bilayer of hepatocyte membranes leading to hepatocellular death and centrilobular liver necrosis.<sup>1</sup> Renal failure has been shown to coexist with liver toxicity in overdose.

The antidote to APAP poisoning is *N*-acetylcysteine (NAC), a precursor of glutathione, which in addition to increasing glutathione stores may also enhance sulfate conjugation of any unmetabolized APAP. NAC functions as an antioxidant and also has an indirect vasodilatory effect by increasing local nitric oxide (NO) concentrations. The vasodilatory effect on microcirculation enhances local oxygen delivery to peripheral tissues, and thus decreases morbidity and mortality even in established hepatotoxicity.<sup>1</sup>

Correspondence to: Aleksandra Niedzwiecki, 1260 Memorex Drive, Santa Clara, CA 95050, USA. Email: author@drath.com

A unique nutrient formulation (NM) containing among other nutrients, NAC, ascorbic acid and green tea extract has previously been demonstrated to exhibit a broad spectrum of pharmacological, therapeutic, cardio vascular and chemoprotective properties.<sup>3-6</sup> This study was undertaken to determine whether NM is useful in prevention of APAP-induced hepatic and renal damage.

## Materials and methods

### Materials

Acetaminophen powder obtained from Sigma Chemical Co. (St. Louis, Missouri, USA) was diluted in saline to 25 mg/ml. Stock solution of the nutrient mixture (NM) was composed of the following in the ratio indicated: vitamin C (as ascorbic acid and as Mg, Ca, and palmitate ascorbate) 700 mg; L-lysine 1000 mg; L-proline 750 mg; L-arginine 500 mg; N-acetyl cysteine 200 mg; standardized green tea extract (80% polyphenol) 1000 mg; selenium 30 µg; copper 2 mg; manganese 1 mg.

### Animals

Male ICR mice, free of murine viruses, bacteria, and parasites, approximately 6 weeks of age on arrival, were purchased from Simonsen Laboratories, Gilroy, California and maintained in microisolator cages under pathogen-free conditions on a 12-h light/12-h dark schedule for a week. All animals were cared for in accordance with institutional guidelines for the care and use of experimental animals.

### Experimental design

After 1 week of isolation, 7-week-old male ICR mice ( $n = 20$ ), weighing 30–32 g were divided into four groups of five mice each (A–D). Groups A and C mice were fed a regular mouse chow diet for 2 weeks, while groups B and D mice were fed the regular diet supplemented with 0.5% NM (w/w) during that period. During the study, the mice consumed, on the average, 4 g of their respective diets per day. Thus, the supplemented mice received approximately 20 mg of NM per day. Subsequently, saline was administered i.p. to group A mice (control) and group B mice; APAP (600 mg/kg body weight) was administered i.p. to groups C and D. All animals were killed 24 h after APAP or saline administration. Mice were anesthetized with isoflurane USP (Abbott Labs, Chicago, Illinois, USA), the abdominal cavity was opened, and ~1 ml of blood was removed by cardiac puncture from each

mouse. Blood was allowed to clot and blood samples were spun at 3000 rpm (1509 g) for 5 min at 4 °C. The samples were stored at –80°C until they were sent for renal and hepatic enzyme analysis. Kidneys and livers were excised from the mice, weighed and processed for histology.

### Histology

Tissue samples were fixed in 10% buffered formalin. All tissues were embedded in paraffin and cut at 4–5 microns. Sections were deparaffinized through xylene and graduated alcohol series to water and stained with hematoxylin and eosin (H&E) for evaluation using a standard light microscope.

### Serum analyses

All chemistry tests for the liver [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase] and kidney (creatinine and BUN) functions were run on a Hitachi 747 Chemistry Analyzer with Boehringer Mannheim Corporation (Rosche, 9115 Hague Road, Indianapolis, Indiana 46250) reagents.

### Statistical analysis

The results were expressed as mean  $\pm$  SD values for the groups. Data were analyzed by ANOVA one-way variance and by independent sample *t*-test.

## Results

### Liver histology and gross appearance

Although gross analysis of the livers from all mice showed no apparent abnormality, histological evaluation showed significant differences in the groups. Acetaminophen treatment caused significant centrilobular necrosis in the livers of unsupplemented (group C) animals, as shown in Figure 1C, while dietary supplementation with 0.5% NM prior to acetaminophen treatment prevented the magnitude of these alterations in group D mice (Figure 1D). Control (group A) and NM 0.5% diet supplemented (group B) mice that received saline instead of acetaminophen injections, showed liver histology within normal limits, as shown in Figure 1A,B, respectively.

### Renal histology and gross appearance

Although gross analysis of the livers from all mice showed no apparent abnormality, histological evaluation demonstrated significant differences between

