Human & Experimental Toxicology (2008) 27: 223–230
www.het.sagepub.com

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

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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.[1] 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.[2]

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 conjugative 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.[1] 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.[1]

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10.1177/0960327108090276

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.[3-6] 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 isoflurance 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 ± 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

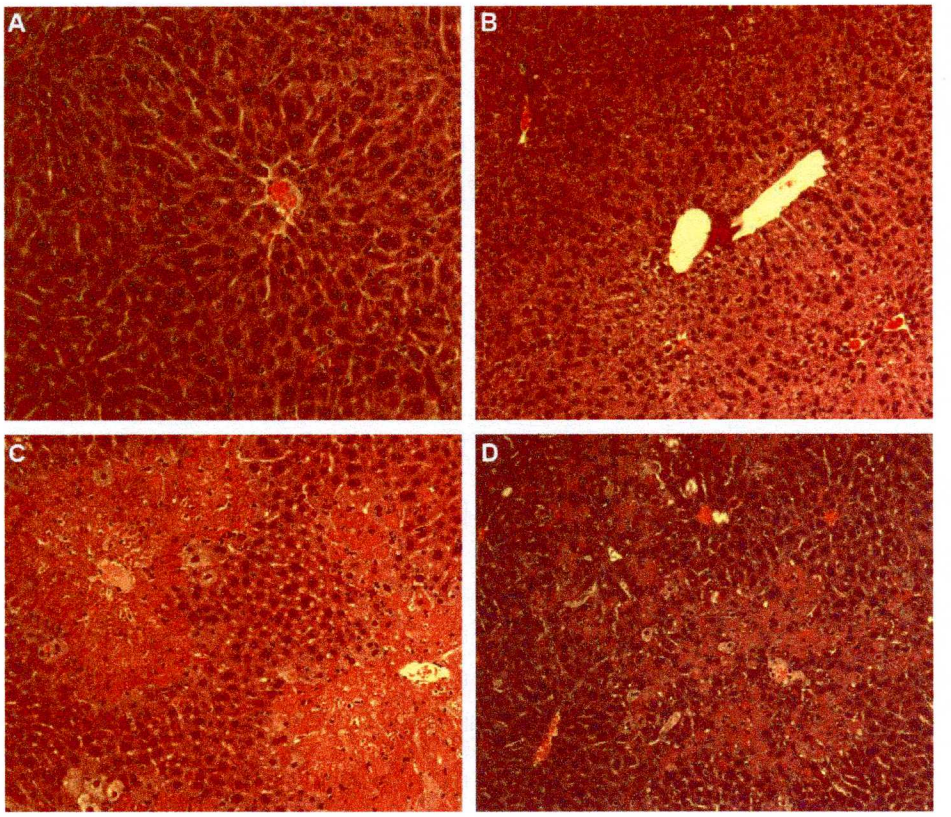

Figure 1 (A) Sample liver section from untreated (Control group A) ICR mice H&E 200x. Liver section within normal limits. (B) Sample liver section from NM-supplemented (group B) ICR mice H&E 200x. Liver section within normal limits. (C) Sample liver section from ICR mice treated with APAP 600 mg/kg (group C) H&E 200x. APAP caused significant centrilobular necrosis in unsupplemented mice, as evidenced in small dark pyknotic nuclei. (D) Sample liver section from NM 0.5% supplemented ICR mice treated with APAP 600 mg/kg 200x (group D). NM treatment provided some protection; liver section shows mild centrilobular necrosis.

the groups. The renal tissue of mice not treated with APAP (untreated control and the NM-treated group) (Figure 2A) showed normal histology. The kidneys of APAP-intoxicated mice (group C) showed severe acute damage to the glomerulus and proximal tubule, as shown in Figure 2B, in contrast to the kidney tissue of mice supplemented with NM prior to APAP injection (group D), which demonstrated less pathology than group C animals, as shown in Figure 2C.

Liver serum markers

Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in ICR mice not treated with APAP (control group A and NM-supplemented group B) were comparable, significantly elevated in APAP-treated mice (group C) and significantly reduced in mice supplemented with NM prior to APAP injection. Serum ALT, AST, and alkaline phosphate values were shown in Table 1. ANOVA one-way variance analysis demonstrated that serum ALT, AST, and alkaline phosphatase results reached statistical significance of $P < 0.001$. Serum ALT values for groups A and B were 25 ± 3 and 28 ± 2 IU/l. Serum ALT was increased to $18{,}926 \pm 2500$ IU/l (75,704% of control) with APAP administration, as shown in Figure 3A. Pretreatment of mice with the NM diet prior to APAP administration, reduced the effect of APAP by 82% ($P = 0.0001$), demonstrating an ALT level of 3397 ± 1012 IU/l. Serum AST for groups A and B were 55 ± 8 and 76 ± 8 IU/l. Serum AST increased to 9444 ± 2220 IU/l (16,924% of control) with APAP administration, as shown in Figure 3B. Pretreatment of mice with the NM diet prior to APAP administration reduced the effect of APAP by 87% ($P < 0.0001$) to 1203 ± 530 IU/l. Serum alkaline phosphatase for groups A and B were 178 ± 109 and 112 ± 8 IU/l, respectively. Serum alkaline phosphatase was increased to 251 ± 13 IU/l (141% of control) with

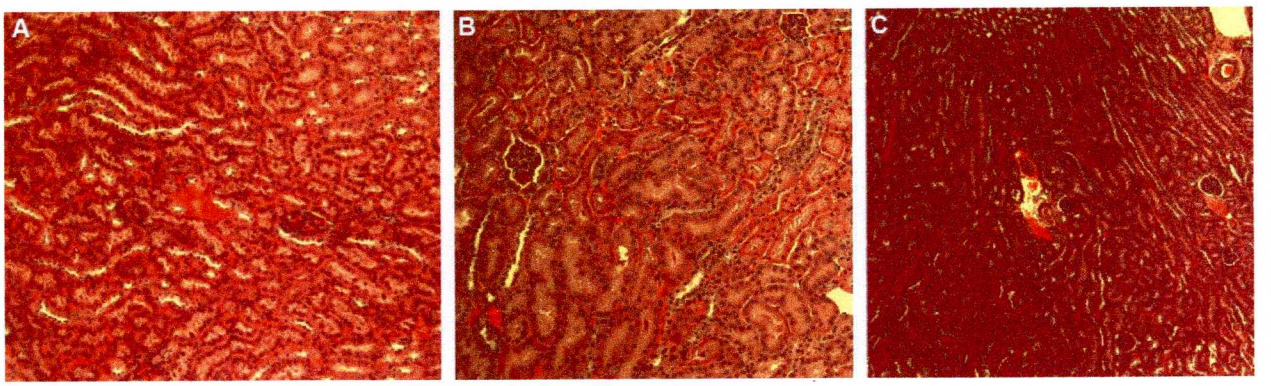

Figure 2 (A) Sample kidney section from untreated (control group A) ICR mice 200x. Kidney section shows no pathology. (B) Sample kidney section from ICR mice treated with APAP 600 mg/kg (group C). APAP caused acute damage to the glomerulus and proximal tubule 200x. (C) Sample kidney section from NM 0.5% supplemented ICR mice treated with APAP 600 mg/kg (group D) 200x. Kidney section shows no pathology.

APAP administration, as shown in Figure 3C. Treatment of mice with the NM diet prior to APAP reduced serum level of alkaline phosphatase to 134 ± 14 IU/l (53% of that with APAP; $P < 0.0001$).

Renal serum markers

Serum markers of kidney: BUN (Figure 4A), creatinine (Figure 4B), and BUN/creatinine ratio (Figure 4C) were markedly increased in the APAP-treated group, in contrast to the control and NM-supplemented groups. The NM-supplemented mice treated with APAP (group D) exhibited biochemical parameters similar to the control group. Summary of renal serum levels for groups was shown in Table 2. ANOVA one-way variance analysis demonstrated that serum BUN, creatinine, and BUN/creatinine results reached statistical significance of $P = 0.005$, 0.048, and 0.045, respectively. Serum BUN values for groups A and B were 22.2 ± 0.5 and 17 ± 4 mg/dl. Serum BUN was increased by 27 to 49 ± 11 mg/dl (220% of control) with APAP administration (group C). Treatment of mice with the NM diet prior to APAP (group D) prevented the effect of APAP, demonstrating BUN value of 18.6 ± 0.07 mg/dl (38%, $P = 0.0003$ of that observed in APAP treatment). Serum creatinine values for groups A and B were 0.52 ± 0.04 and 0.48 ± 0.06 mg/dl.

Serum creatinine was increased by 0.23 mg/dl to 0.75 ± 0.13 mg/dl (144% of control) with APAP administration (group C). Treatment of mice with the NM diet prior to APAP administration (group D) prevented the effect of APAP, demonstrating a level within normal limits, 0.44 ± .02 mg/dl (42% of that with APAP, $P = 0.0005$). BUN/creatinine ratios for groups A and B were 44 ± 4 and 47 ± 5, respectivley. Dietary pretreatment of ICR mice with NM (group D) reduced mean BUN/creatinine ratio by 32% ($P < 0.0001$) compared with that seen in ICR mice receiving unsupplemented diet prior to APAP administration (group C).

Mean body weights

Mean body weights of mice did not differ significantly between groups ($P = 0.63$). Initial body weights of mice ranged between 30 and 32 g; at sacrifice, mean body weights of groups were: A (control) 30.9 ± 2.5 g, B (NM) 32.2 ± 1.7 g, C (APAP) 31.1 ± 1.9 g, and D (APAP + NM) 31.5 ± 1.0 g.

Liver and kidney weights

APAP treatment (groups C and D) resulted in a marked increase in mean liver (Figure 5A) and kidney (Figure 5B) weights. Mean liver weight of ICR

Table 1 Mean ± SD values of liver functional enzymes among ICR mouse groups

Groups	*APAP or saline injection*	*2-week diet*	*AST (IU/l)*	*ALT (IU/l)*	*Alkaline phosphatase (IU/l)*
A ($n = 5$)	Saline	Regular mouse chow	55 ± 8	25 ± 3	178 ± 10
B ($n = 5$)	Saline	0.5% NM supplemented	76 ± 8	28 ± 2	112 ± 8
C ($n = 5$)	APAP 600 mg/kg	Regular mouse chow	9444 ± 2220	18,926 ± 2500	251 ± 13
D ($n = 5$)	APAP 600 mg/kg	0.5% NM supplemented	1203 ± 530	3397 ± 1012	134 ± 14

ANOVA one-way variance analysis for ALT, AST, and alkaline phosphatase reached significance level of $P < 0.001$.

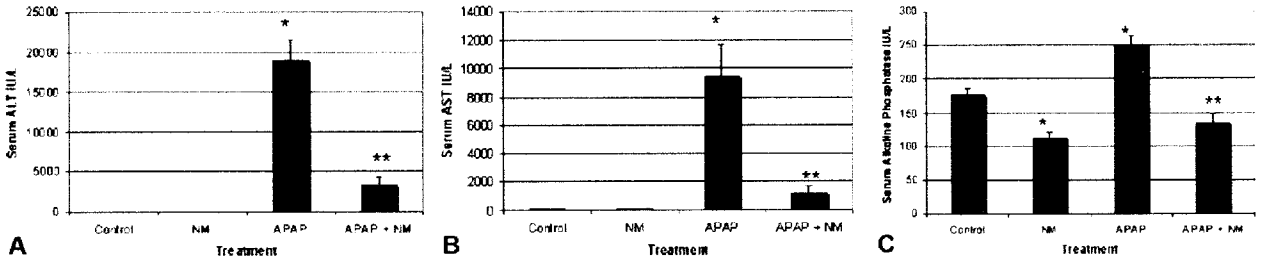

Figure 3 (A) Effect of APAP 600 mg/kg administered to NM-supplemented and unsupplemented ICR mice on serum alanine aminotransferase (ALT). ALT in ICR mice not treated with APAP (control group A and NM supplemented group B) were comparable. Treatment with APAP (group C) resulted in marked increase in ALT (to 18,926 IU/l). Supplementation with NM prior to APAP injection (group D) significantly inhibited the effect of APAP (by 82%, $P = 0.0001$). *$P < 0.0001$ with respect to the control group; **$P = 0.0001$ with respect to APAP group. (B) Effect of APAP 600 mg/kg administered to NM-supplemented and unsupplemented ICR mice on aspartate aminotransferase (AST). AST in ICR mice not treated with APAP (control group A and NM supplemented group B) were comparable. AST was increased to 9444 IU/l with APAP administration (group C). Treatment of mice with the NM diet prior to APAP (group D) inhibited the effect of APAP by 87% ($P < 0.0001$). *$P < 0.0001$ with respect to the control group; **$P < 0.0001$ with respect to APAP group. (C) Effect of APAP 600 mg/kg administered to NM-supplemented and unsupplemented ICR mice on alkaline phosphatase. Alkaline phosphatase in ICR mice not treated with APAP (control group A and NM-supplemented group B) were comparable. Serum alkaline phosphatase was increased to 251 IU/l (141% of control) with APAP administration (group C). Treatment of mice with the NM diet prior to APAP (group D) inhibited the effect of APAP by 47% ($P < 0.0001$). *$P < 0.0001$ with respect to the control group; **$P < 0.0001$ with respect to APAP group.

mice increased by 36%, $P = 0.0003$ over control liver weight with APAP administration (2.45 ± 0.18 g for group C and 2.5 ± 0.2 g for group D). Mean liver weights of control group A and NM group B were 1.8 ± 0.15 g.

Mean kidney weights of ICR mice increased by 40%, $P = 0.0001$ over control kidney weight with APAP administration (0.73 ± 0.1 g for group C and 0.7 ± 0.1 g for group D). Mean kidney weights of control group A and NM group B were 0.52 ± 0.05 and 0.50 ± 0.06 g. Pretreatment with NM did not affect kidney weights of APAP-treated mice.

Discussion

The results of the present study show that pretreatment for 2 weeks with a diet supplemented with 0.5% of the nutrient mixture reduced hepatic and renal damage in ICR mice from administration of a toxic dose (600 mg/kg body weight) of acetaminophen. APAP treatment caused significant hepatic centrilobular necrosis and marked increases in hepatic serum markers AST, ALT, and alkaline phosphatase in unsupplemented mice; supplementation with NM prior to APAP administration

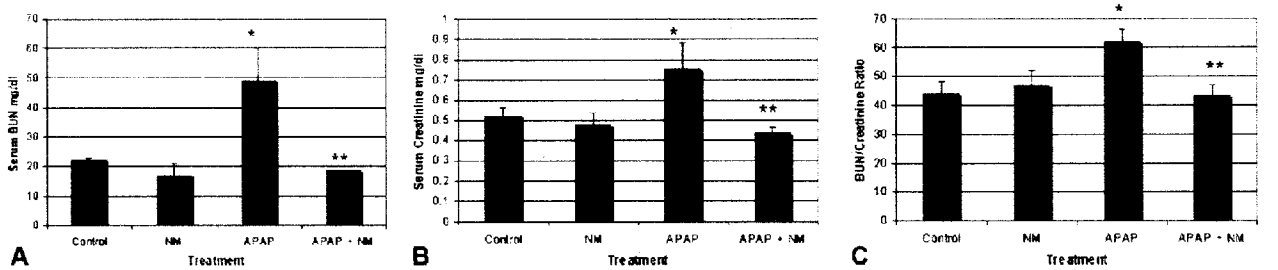

Figure 4 (A) Effect of APAP and NM on serum BUN levels in ICR mice. Serum BUN in ICR mice not treated with APAP (control group A and NM-supplemented group B) were comparable. BUN was increased to 49 IU/l (220% of control) with APAP administration (group C). Treatment of mice with the NM diet prior to APAP (group D) reversed the effect of APAP by inhibiting the effect of APAP by 62% ($P = 0.0003$). *$P = 0.0006$ with respect to the control group; **$P = 0.0003$ with respect to APAP group. (B) Effect of APAP and NM on serum creatinine levels in ICR mice. Serum creatinine in ICR mice not treated with APAP (control group A and NM-supplemented group B) were comparable. Creatinine was increased to 0.75 mg/dl (144% of control) with APAP administration (group C). Supplementation of mice with the NM diet prior to APAP administration (group D) inhibited the effect of APAP by 58% ($P = 0.0005$). *$P = 0.005$ with respect to the control group; **$P = 0.0008$ with respect to APAP group. (C) Effect of APAP and NM on serum BUN/creatinine ratio in ICR mice. Serum BUN/creatinine ratios in ICR mice not treated with APAP (control group A and NM supplemented group B) were comparable. Dietary pretreatment of ICR mice with 0.5% NM (group D) inhibited APAP-induced (group C) elevation of mean BUN/creatinine ratio by 32% ($P < 0.0001$). *$P = 0.0001$ with respect to the control group; **$P < 0.0001$ with respect to APAP group.

Table 2 Mean ± SD values of renal functional enzymes among ICR mouse groups

Groups	*APAP or saline injection*	*2-week diet*	*BUN (mg/dl)*	*Creatinine (mg/dl)*	*BUN/creatinine*
A ($n = 5$)	Saline	Regular mouse chow	22.2 ± 0.5	0.52 ± 0.04	44 ± 4
B ($n = 5$)	Saline	0.5% NM supplemented	17 ± 4	0.48 ± 0.06	47 ± 5
C ($n = 5$)	APAP 600 mg/kg	Regular mouse chow	49 ± 11	0.75 ± 0.13	62 ± 4
D ($n = 5$)	APAP 600 mg/kg	0.5% NM supplemented	18.6 ± 0.07	0.44 ± 0.02	43.2 ± 4

ANOVA one-way variance analysis for BUN, creatinine, and BUN/creatinine reached significance level of $P = 0.005$, 0.048, and 0.045 respectively.

reduced the histological changes in the liver as well as maintained the AST, ALT, and alkaline phosphatase levels within normal limits. Elevated ALT and AST levels reflect hepatocellular inflammation, damage and necrosis. Increased levels of alkaline phosphatase are associated with biliary tract damage and inflammation. Renal histology of acetaminophen-intoxicated mice showed severe glomerular and proximal tubule damage and significantly increased creatinine and BUN levels. NM dietary supplementation prior to APAP injection reduced APAP-induced tissue damage as well as prevented the APAP-induced elevation of renal serum marker levels.

The nutrient mixture tested was formulated based on targeting different physiological processes involved in a wide spectrum of pathological conditions at the cellular level. Diverse antioxidants have been shown to prevent APAP-induced hepatotoxicity in mice.[7] Vitamin C has been shown to provide a pronounced protective effect when given in sufficient doses. Peterson and Knodell[8] found that a 1000-mg/kg body size dose of vitamin C given either 1 h before or 1 h after a toxic dose of acetaminophen had a pronounced protective effect. However, when used in lower doses (maximal dose 200 mg/kg body weight), vitamin C did not increase the survival rate of mice given potentially lethal doses of acetaminophen.[9] A 600-mg/kg body weight dose of ascorbyl palmitate was able to significantly protect mice from acetaminophen-induced liver damage, while regular vitamin C did not have that protective effect at the same dose.[10,11]

Green tea polyphenols have also been shown to prevent liver injury from APAP-induced hepatoxicity.[7] Green tea polyphenols have also shown protective effects against administration of other toxic chemicals. Pretreatment with EGCG (Epigallocatechin Gallate) led to a dose-dependent decrease in all of the histological and biochemical variables of liver injury observed in the carbon tetrachloride-treated (20 µl CCl_4/kg weight) mice.[12] Green tea polyphenols reduced the severity of liver injury with lower concentrations of lipid peroxidation and proinflammatory NO-generated mediators. Hasegawa *et al.*[13] reported pretreatment of male rats with 2% green tea as drinking water for 2 weeks prior to a single i.p. injection of the carcinogen 2-nitropropane (2NP) at a dose of 100 mg/kg body weight provided effective protection against induction of hepatic degenerative changes by 2NP. Green tea effectively blocked oxidative DNA damage to the liver as well as hepatotoxicity in rats treated with 2NP.[13]

Another component of the nutrient mixture important for protection of the liver from APAP tox-

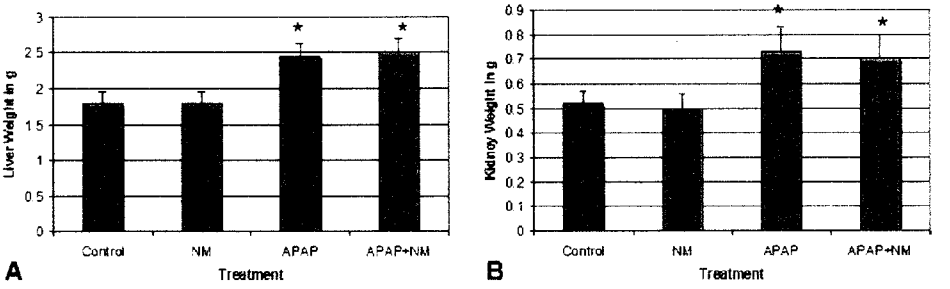

Figure 5 (A) Effect of APAP administration on mean liver weight of ICR mice. Mean weight of ICR mice increased by 36%, $P = 0.0003$ over control liver weight with APAP administration (2.45 ± 0.18 g for group C, and 2.5 ± 0.2 g for group D). Mean liver weights of control group A and NM group B were 1.8 ± 0.15 g. Pretreatment with NM did not affect liver weights of APAP-treated mice. *$P = 0.0003$ with respect to the control group. (B) Effect of APAP administration on mean kidney weight of ICR mice. Mean kidney weight of ICR mice increased by 40%, $P = 0.0001$ over control kidney weight with APAP administration (0.73 ± 0.1 g for group C, and 0.7 ± 0.1 g for group D). Mean kidney weights of control group A and NM group B were 0.52 ± 0.05 and 0.50 ± 0.06 g. Pretreatment with NM did not affect kidney weights of APAP-treated mice. *$P = 0.0001$ with respect to the control group

icity is *N*-acetyl cysteine, which, as mentioned earlier, is used as an antidote for APAP toxicity by increasing glutathione stores, providing a glutathione substitute, and directly conjugating with NAPQI. In addition, NAC acts as an antioxidant inhibiting neutrophil accumulation and reducing APAP-induced damage. NAC is administered p.o. with a loading dose of 140 mg/kg followed by maintenance doses of 70 mg/kg every 4 h for a total of 17 doses unless APAP measurement of the patient is at a nontoxic level[1,14]; liver protection has been shown to be equally effective when NAC treatment is initiated within 0, 4 or 8 h of APAP toxic ingestion.[14] The low dose of NAC provided to the supplemented ICR mice in our study, 3.5 mg/kg, demonstrates the efficacy of using NAC in combination with other nutrients to achieve the protective effect of mega doses of NAC alone. Arginine, another component of NM is a precursor of NO which has vasodilatory effect on microcirculation, enhancing local oxygen delivery to peripheral tissue.[15,16]

The role of matrix metalloproteinases (MMPs) in APAP-induced hepatic centrilobular necrosis was reported by Ito *et al.*[17] Increased levels of MMP-2 and MMP-9 in mice during APAP intoxication were shown to be associated with hepatocellular damage and with hepatic microcirculatory dysfunction, including impaired sinusoidal perfusion and infiltration of erythrocytes into the space of Disse. Pretreatment of mice with an MMP inhibitor minimized hepatic microvascular injury after treatment with acetaminophen 600 mg/kg body weight, suggesting use of MMP inhibitors for treatment of APAP toxicity. Our previous studies with a wide variety of cell types have shown that NM inhibits MMP-2 and MMP-9 secretions and cellular invasion through the ECM.[3–6]

ECM integrity is dependent upon adequate collagen formation and its stability. In this aspect ascorbic acid and the amino acids lysine and proline are necessary for the formation and optimum structure of collagen fibers.[18] Manganese and copper are also essential cofactors in collagen formation process. Collagen stability can be controlled by lysine[18] and also by *N*-acetyl cysteine through its inhibitory effect on MMP-9 activity.[19] Selenium has been shown to interfere with MMP expression and tumor invasion, [20] as well as migration of endothelial cells through ECM.[21] Green tea extract has shown to be a promising agent in controlling angiogenesis, metastasis, and other aspects of cancer progression through inhibition of MMPs and cellular invasion.[22]

Based on our own research and published data, we postulated that metabolic effects of a combination of ascorbic acid, lysine, proline, green tea extract, arginine, *N*-acetyl cysteine, selenium, copper and manganese would result from their synergy.[23,24] For example, we found that a combination of ascorbic acid, lysine and proline used with EGCG enhanced the anti-invasive activity of 20 μg/ml EGCG to that of 50 μg/ml.[21] Thus by including nutrients like *N*-acetyl cysteine, arginine, selenium, manganese, and copper in addition to ascorbic acid, proline, lysine, and EGCG, we could obtain significant reduction in cell invasion at a much lower concentration of EGCG.

In conclusion, this study demonstrated that pretreatment for 2 weeks with a diet supplemented with 0.5% NM reduced hepatic and renal damage in ICR mice from administration of a toxic dose of acetaminophen. Supplementation of ICR mice with dietary NM reduced the pathological, histological changes in the liver and kidney, as well as prevented APAP-induced elevation of hepatic and renal serum markers. Although clinical studies are warranted, these results suggest that individuals who use APAP to control chronic pain would benefit from ingestion of the nutrient mixture to protect against APAP-induced liver and kidney damage.

Acknowledgments

Consulting pathologist Dr. Michael V. Slayter, DVM, MPVM provided the histology slides. Liver and kidney enzyme analyses were provided by IDEXX Laboratories Inc.

The research study was funded by Dr. Rath Health Foundation (Plantation, Florida, USA), a non-profit organization.

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