



# A Nutrient Mixture Suppresses Carbon Tetrachloride Induced Hepatic Toxicity in ICR Mice

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## Abstract

### **Introduction:**

Recent studies from our laboratory indicated that a unique nutrient formulation consisting of lysine, proline, ascorbic acid and green tea extract (NM) demonstrates a broad spectrum of pharmacological, therapeutic, cardiovascular and chemoprotective properties.

### **Objective:**

We examined the effect of NM in mice treated with carbon tetrachloride (CT), a model of liver injury in which free radical, oxidative stress and cytokine production are closely linked.

### **Materials and Methods:**

Six-week-old male ICR mice were divided into four groups of five animals each; Group A received corn oil and was used as a normal control. Group B mice received CT (25  $\mu$ L/kg, in corn oil, ip), group C mice were fed NM (0.5%) for two weeks, and group D mice were dosed with CT (25  $\mu$ L/kg, in corn oil, ip) after feeding NM (0.5%) for two weeks. All animals were sacrificed after 24 hrs. Blood was withdrawn by cardiac puncture and serum was collected for markers of liver and kidney. Liver and kidney were excised for histology

### **Results:**

Administration of CT to group B mice resulted in marked increase in serum AST and ALT levels (markers for liver). Serum ALT and AST in groups A and C were of low magnitude and were comparable, whereas in group D animals these enzymes were significantly reduced. CT had no effect on renal markers (BUN, creatinine and BUN/creatinine ratio). CT administration caused an intense degree of liver necrosis that was less severe in the NM fed group D.

### **Conclusions:**

These results indicate that NM could be a useful supplement in treating liver diseases due, in part, to its antioxidant properties and ability to scavenge CT-associated free radicals.

## Comment:

Liver disease and toxicity is common, especially with many drug treatments. Carbon tetrachloride (CT)-induced liver injury in mice is an excellent model to evaluate the effectiveness of agents on inhibition or reversal of liver damage. A unique nutrient formulation consisting primarily of lysine, proline, ascorbic acid and green tea extract (NM) has demonstrated a broad spectrum of pharmacological, therapeutic, cardiovascular and chemoprotective properties. We examined the effect of a NM supplemented diet on liver and kidney tissue and serum markers in ICR mice dosed with CT. Administration of CT to mice on a regular diet resulted in marked increase in serum AST and ALT levels (markers for liver). In contrast, serum ALT and AST levels in NM-supplemented mice were of low magnitude and comparable to control mice (not dosed with CT). CT administration caused an intense degree of liver necrosis that was less severe in the NM fed group. These results are significant as they indicate that NM has potential in treating and preventing liver disease.

### A NUTRIENT MIXTURE SUPPRESSES CARBON TETRACHLORIDE INDUCED HEPATIC TOXICITY IN ICR MICE

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#### Introduction:

Recent studies from our laboratory indicated that a unique nutrient formulation consisting of lysine, proline, ascorbic acid and green tea extract (NM) demonstrates a broad spectrum of pharmacological, therapeutic, cardiovascular and chemoprotective properties.

#### Objective:

We examined the effect of NM in mice treated with carbon tetrachloride (CT), a model of liver injury in which free radical, oxidative stress and cytokine production are closely linked.

#### Materials and Methods:

1. Six-week-old male ICR mice were divided into four groups of five animals each.

- Control - received corn oil
- CT - received CT (25 µL/kg, in corn oil, ip)
- NM - were fed NM (0.5%) for two weeks
- CT + NM - were dosed with CT (25 µL/kg, in corn oil, ip) after being fed NM (0.5%) for two weeks.

2. All animals were sacrificed after 24 hrs. Blood was withdrawn by cardiac puncture and serum was collected for markers of liver and kidney.

3. Liver and kidney were excised for histology.

#### 4. Composition of Nutrient Mixture (NM)

Nutrient	Per Stock Solution
Ascorbic acid (ascorbic acid and ascorbyl-2-pyruvate succinate)	270 mg
Lysine	1000 mg
Proline	250 mg
L-Arginine	500 mg
N-Acetyl Cysteine	200 mg
Standardized Green Tea Extract (EGCG enriched)	1000 mg
Selenium	50 µg
Copper	5 mg
Manganese	5 mg

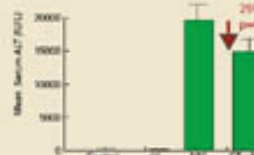
#### Results:

1. Administration of carbon tetrachloride (CT) to ICR mice resulted in marked increase in serum AST and ALT levels (liver markers). Serum ALT and AST in control and NM groups were of low magnitude and were comparable, whereas in the NM + CT animals these enzymes were significantly reduced (Figures 1A,B).

Figure 1A - Hepato-protective effect of NM on CT induced toxicity in male ICR mice: serum aspartate aminotransferase (AST/SGOT)



Figure 1B - Hepato-protective effect of NM on CT induced toxicity in male ICR mice: serum alanine aminotransferase (ALT/SGPT)



2. Carbon tetrachloride administration caused significant liver necrosis in unsupplemented animals (Figure 2C), while NM reduced these alterations in the CT +NM group (Figure 2D). Control and NM fed mice had normal liver histology (Figures 2A,2B).

Figure 2A - Sample liver section from control mice.



Figure 2B - Sample liver section from NM fed mice.



Figure 2C - Sample liver section from CT-treated mice.



Figure 2D - Sample liver section from CT + NM-treated mice.



3. CT had no significant effect on renal markers: BUN (Figure 3A), creatinine (Figure 3B), and BUN/creatinine ratio (Figure 3C). CT had minimal effect on kidney tissue (histology not shown).

Figure 3A - Effect of NM and CT treatment of ICR mice on serum BUN

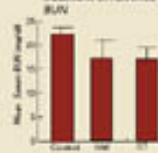


Figure 3B - Effect of NM and CT treatment of ICR mice on serum creatinine

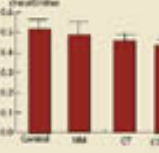
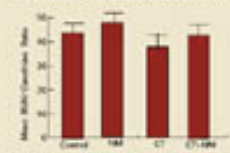


Figure 3C - Effect of NM and CT treatment of ICR mice on serum BUN/creatinine ratio



#### Conclusions:

These results indicate that the nutrient mixture tested (NM) has potential in treating liver diseases due, in part, to its antioxidant properties and ability to scavenge CT-associated free radicals.