HEPASIL DTX™ INCREASES BOTH ANTIOXIDANT AND DETOXIFICATION CAPACITY BY BOOSTING GLUTATHIONE AND VITAMIN C LEVELS

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INTRODUCTION

The liver is the major detoxification organ in the body, as the primary site of Phase I and Phase II detoxification enzymes. Phase I includes the cytochrome P450 family of enzymes. These serve as a first line of defense and initially oxidize toxins. Unfortunately, this initial oxidation can convert compounds into more toxic metabolites. Phase II detoxification enzymes can further modify Phase I metabolites by conjugating them with water-soluble molecules. This step renders them less toxic and as a result, more easily excreted from the body. If not modified by Phase II enzymes, Phase I metabolites can damage biomolecules; therefore, it is essential that this detoxification system continually favors Phase II reactions. Furthermore, it is imperative that the levels and efficiency of Phase II detoxification enzymes, as well as the pool of conjugation substrates, remain high to maintain optimal liver detoxification capacity. Two important antioxidant and conjugation substrates involved in these detoxification pathways are glutathione and vitamin C.

Glutathione (GSH) is the most abundant low-molecular weight, water-soluble antioxidant in the body and plays a major role in the detoxification process. Unfortunately, GSH is inefficiently absorbed from the diet and must be synthesized in the body by specific Phase II enzymes. Under normal conditions, GSH production is low; however, during times of toxicological insult, the body increases production of GSH by up-regulating Phase II enzymatic machinery. Interestingly, a number of phytochemicals including broccoli extract, Milk thistle, and alpha-lipoic acid have also been shown to up-regulate Phase II enzymes—including those that synthesize GSH[1-5].

Vitamin C is an important first line of defense in protecting biomolecules from oxidative damage, especially in circulation. Moreover, recent scientific evidence suggests that vitamin C may also play a role in the removal of toxins[6-11]. The concentration of vitamin C in the body is tightly regulated by intestinal absorption from the diet and recycling by the kidneys[12, 13]. Because of this tight regulation, it had previously been thought that circulating vitamin C levels could only be increased by supplementing with vitamin C. However, it has recently been shown that both circulating and tissue levels of vitamin C can be increased by certain phytochemicals even in the absence of vitamin C supplementation[12, 13].

To determine the potential consequences of increased GSH and vitamin C levels, two functional experiments were utilized. First, we conducted a glutathione S-transferase (GST) activity assay. GSTs are a class of enzymes that catalyze the conjugation of reduced GSH, via the sulfhydryl group, to various electrophilic compounds[14]. This activity assists in the removal and excretion of a number of compounds including oxidized biomolecules, toxins, and other xenobiotics. The second experiment assessed the antioxidant status of the study participants with an assay developed by USANA Health Sciences—the Plasma/Serum Antioxidant Reserve (SAR)[15]—a powerful *ex vivo* measurement of the antioxidant capacity of human blood.

While individual nutrients and phytochemicals have been shown to increase both GSH levels and vitamin C, to date, it has never been shown that the combination of these nutrients and phytochemicals can synergistically boost both GSH and vitamin C levels simultaneously. Thus, the purpose of this study was to assess the effectiveness of Hepasil DTX™ in increasing both GSH and vitamin C levels.

MATERIALS AND METHODS

Study Design

This was a double-blind, placebo-controlled study. Fifteen healthy volunteers between the ages of 24 and 45 completed the study (N = 7 for the treatment group and N = 8 for the placebo group). Hepasil DTX™ and placebo were provided by USANA Health Sciences. Subjects were instructed to take the recommended dose of 3 tablets per day for 4 weeks. On days 1 and 28, blood samples were drawn at 0, 2, 4, and 8 hours. A baseline blood draw was also taken on day 14. Blood samples were flash-frozen (whole blood, serum, and plasma [+/- acidification]) in liquid nitrogen before storage at -120˚C.

Determination of Plasma GSH and Vitamin C

Glutathione analytes were separated by injecting 1 µL of the prepared sample into an Agilent (Series 1200) HPLC using a Phenomenex C18 column. Method conditions were: 0-7 minutes at 0.5 mL/min with 0.03% formic acid in water and 8-15 minutes 0.5 mL/min with 2-propanol. Both reduced glutathione (GSH) and oxidized glutathione (GSSG) were detected on an Agilent tandem mass spectrometer (Series 6410, Model G6410A) using an electrospray (ES) source. GSH concentrations were determined relative to authentic standards and expressed as total soluble glutathione (GSH + 2GSSG) relative to placebo group.

Vitamin C was separated by injecting 7 µL of the prepared sample into the aforementioned HPLC. Method conditions were: 1-7 minutes at 0-5 mL/min in 0.03% formic acid and 8-15 minutes at 0.5 mL/min in 2-propanol. Vitamin C was detected on a Hewlett-Packard UV detector (254 nM; Series 1050). Vitamin C concentrations were determined relative to authentic standards and expressed relative to the placebo group.

Determination of GST Activity and SAR

A small aliquot of whole blood was diluted and assayed for GST activity using a GST Assay Kit (#703302 Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions.

A small aliquot of serum was used for SAR. Oxidation of serum was initiated by addition of 1.0 mmol SIN-1 chloride. After incubation for 4 hours at 37˚C, the oxidation reaction was stopped by addition of BHT and EDTA. The induced 8-isoprostanes were then measured using an ELISA kit (8-*iso* Prostaglandin $F_{2\alpha}$ Kit, Cayman Chemical, Ann Arbor, MI). Calibration, curve fitting, and data analysis was conducted according to the manufacturer's instructions.

RESULTS

Glutathione

- **Hepasil DTX™ acutely, chronically, and acute-on-chronically increased plasma total GSH levels (data not shown).**
- **Hepasil DTX™ increased plasma GSH 2 hours following the first treatment and significantly increased plasma GSH 8 hours after supplementation ($p < 0.05$; data not shown).**
- **A chronic 18.3% increase in plasma GSH was observed, but did not reach statistical significance (data not shown).**
- **Hepasil DTX™ significantly increased plasma GSH 8 hours after supplementation during the Acute-on-Chronic Phase ($p < 0.05$; data not shown).**
- **Plasma GSH levels increased by 74% by the end of the study (data not shown).**

Vitamin C

- **Hepasil DTX™ significantly increased plasma vitamin C as soon as 2 hours following the first treatment and was maintained during the entire Acute Phase ($p < 0.05$; data not shown).**
- **A chronic increase in vitamin C was seen, but did not reach statistical significance (data not shown).**
- **Hepasil DTX™ significantly increased plasma vitamin C concentrations during the Acute-on-Chronic Phase of the study ($p < 0.05$; data not shown).**

Glutathione S-Transferase Activity

- **Hepasil DTX™ acutely, chronically, and acute-on-chronically increased whole blood GST activity (Figures 3A, 3B, and 3C).**
- **GST activity significantly increased 8 hours after supplementation during the Acute Phase and significantly increased 4 and 8 hours during the Acute-on-Chronic Phase ($p < 0.05$; Figures 3A and 3C).**

Serum Antioxidant Reserve

- **Hepasil DTX™ significantly increased SAR by 32% within 2 hours following the first supplementation, and remained significantly elevated for all Acute time points measured ($t = 2, 4, 8$ h; $p < 0.05$; Figure 4A).**
- **A significant chronic 62% increase in SAR was seen by day 28 ($p < 0.05$; Figure 4B).**
- **Hepasil DTX™ significantly increased SAR 8 hours after supplementation during the Acute-on-Chronic Phase ($p < 0.05$; Figure 4C).**

DISCUSSION AND CONCLUSIONS

GSH plays a major role in the overall antioxidant network and also acts as an intermediate/conjugate in the detoxification process. Interestingly, we found a biphasic response in both the Acute and Acute-on-Chronic Phases of the study (data not shown). This can be explained by two distinct mechanisms. The first mechanism is likely due to the N-acetyl-L-cysteine provided by Hepasil DTX™. Cysteine is the rate-limiting substrate in GSH synthesis and has been shown that providing cysteine alone will increase GSH levels. As such, we found a concurrent significant increase in plasma cysteine levels following treatment with Hepasil DTX™ (data not shown). The second mechanism is likely due to an increase in Phase II GSH-synthesizing enzymes over time. Broccoli extract, milk thistle, and alpha-lipoic acid have been shown to up-regulate Phase II enzymes, and likely account for the steady increase in GSH observed during this study. The large Acute-on-Chronic increase in GSH relative to the Acute Phase, is likely due to this steady increase in GSH over the course of the study, in addition to the effects seen during the Acute Phase ($p < 0.05$; data not shown).

To show that GSH-dependent detoxification capacity increased, we went on to measure GST activity in whole blood. GSTs are a class of Phase II enzymes that catalyze the conjugation of reduced GSH to various electrophilic compounds. This activity assists in the removal and excretion of a number of compounds including oxidized biomolecules, toxins, and other xenobiotics. We observed an increase in GST activity in a number of time points following treatment, indicating that Hepasil DTX™ may increase endogenous, GSH-dependent detoxification mechanisms.

Vitamin C is a well-known antioxidant but also has been recently shown to act as an important substrate for detoxification reactions. Relatively recent studies have shown that vitamin C binds to and likely helps facilitate the removal of toxins. The increase found in vitamin C levels is intriguing because the current Hepasil DTX™ formulation does not contain vitamin C. Moreover, study subjects were not taking any nutritional supplements prior to enrollment in the study, nor was there vitamin C in the provided meal (nutritional data not shown). While this result is surprising, there is a scientific rationale for this effect. It has been shown that supplementation with phytochemicals can increase vitamin C transporter levels and ultimately increase vitamin C levels, even in the absence of vitamin C supplementation. Studies are currently underway to examine this effect further.

To show that the observed increases in both glutathione and vitamin C translate into a clinical benefit, we went on to determine the antioxidant capacity of each subject's blood. To do this, we utilized the SAR assay developed by USANA Health Sciences. We found that subjects treated with Hepasil DTX™ were significantly more resistant to oxidative damage than subjects given the placebo.

This proprietary combination of essential nutrients and broccoli extract, milk thistle, and alpha-lipoic acid, not only increases both plasma vitamin C and glutathione levels simultaneously, but also up-regulates the molecular machinery needed to utilize glutathione in the detoxification reactions and significantly increase the overall capacity of the blood to protect against oxidative insult. Cumulatively, these results show that taking the recommended dose of Hepasil DTX™ increased both antioxidant and detoxification capacities by boosting both glutathione and vitamin C levels.

EXPERIMENTAL DESIGN

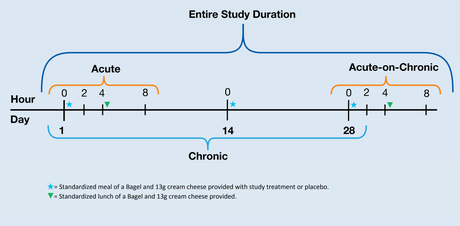

Figure 1. Experimental design. The current study was broken down into three Phases: Acute, Chronic, and Acute-on-Chronic. During the Acute Phase, effects of Hepasil DTX™ were monitored during the first eight hours following the intial treatment (Day 1, hours 0-8). During the Chronic Phase, changes in baseline effects were monitored over the 28-day study (Day 1, hour 0 versus Day 28, hour 0). The Acute-on-Chronic Phase was designed to monitor Acute effects that occurred in addition to those seen Chronically (Day 28, hours 0-8).

GLUTATHIONE AND VITAMIN C ARE INVOLVED IN DETOXIFICATION

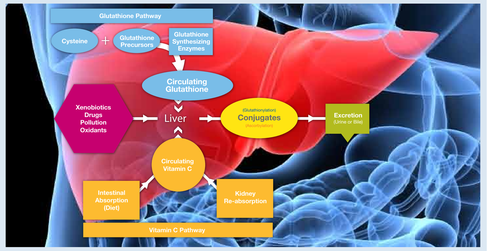

Figure 2. Glutathione and vitamin C are involved in detoxification reactions. In the liver, glutathione-synthesizing enzymes combine cysteine with other glutathione precursors to ultimately form glutathione (blue pathway). Circulating vitamin C levels are determined by intestinal absorption (diet) and recycling in the kidneys (orange pathway). Toxins in the body can be conjugated with either glutathione or vitamin C, ultimately rendering them less toxic and able to be excreted in the urine or bile.

HEPASIL DTX™ INCREASED WHOLE BLOOD GST ACTIVITY

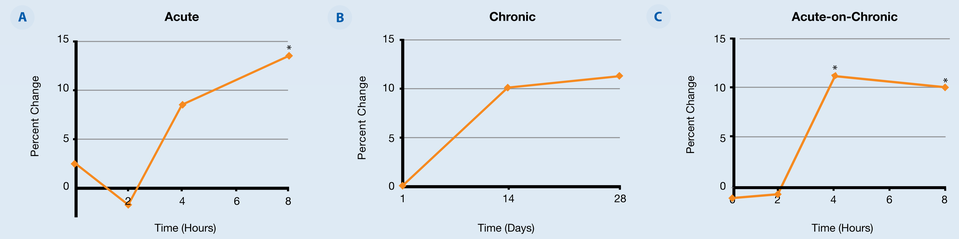

Figure 3. Hepasil DTX™ increased whole blood GST activity. (A) Acute Phase changes in whole blood GST. (B) Chronic phase changes in whole blood GST. (C) Acute-on-Chronic Phase changes in whole blood GST. Asterisks (*) denote statistical significance.

HEPASIL DTX™ INCREASED SERUM ANTIOXIDANT RESERVE

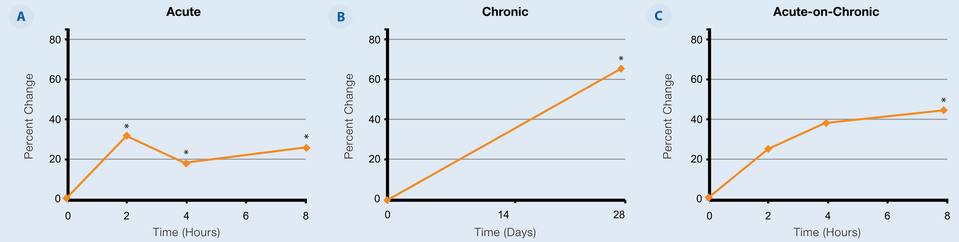

Figure 4. Hepasil DTX™ increased serum antioxidant reserve (SAR). (A) Acute Phase changes in SAR. (B) Chronic phase changes in SAR. (C) Acute-on-Chronic Phase changes in SAR. Asterisks (*) denote statistical significance.

Patent Pending Hepasil DTX™ Hybrid Technology

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