In Vitro Investigations of Increased Biomodulated Activation of Irinotecan to Its Active Metabolite (SN-38) Using Herbal Extracts
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Purpose
The purpose of this study is to evaluate the ability of various herbal extracts to increase the enzymatic bioactivation of the chemotherapeutic prodrug irinotecan (IR) to its active metabolite SN-38 while reducing the production of inactive metabolites.

Methods
Herbal extracts were prepared from commercially available dried plants by heating (60°C) the plants material (10 grams) twice with deionized water (250 mL) for two hours. After heating, the samples were steeped for five hours and the two solutions combined. The resulting aqueous extract was centrifuged and spray dried into a powder. Herbal plants used in this study consisted of lemon balm, blessed thistle, echinacea purpurea, echinacea angustifolia, damiana leaf, and skullcap. Irinotecan (25 μM) was incubated individually with the six herbal extracts at various concentration ranges based on commercially reported doses. Reactions were carried out at 37°C with an NADPH regenerating system and pooled human liver microsomes in triplicate. Reactions were preincubated in a shaking water bath for five minutes and initiated by the simultaneous addition of the substrate and the herbal extract. After 120 minutes, the reactions were quenched with an equal volume of ice cold acetonitrile containing terfenadine as an internal standard. Samples were centrifuged and the supernatant analyzed by LC-MS/MS. Separation was achieved using a Luna C18 column (100 x 2.1 mm) with a gradient elution using an AB Sciex 4000 QTRAP mass spectrometer. The percent increase or decrease in the irinotecan metabolite formation was calculated as a percent as compared to a control reaction without any herbal extract.

Results
Out of the six herbal extracts evaluated, only one (lemon balm), showed a significant increase in SN-38 active metabolite production and a significant decrease in the production of SN-38 glucuronide (SN-38G) and other non-active metabolites within the irinotecan metabolic pathway. The lemon balm extract produced a 60 ± 9% increase in SN-38 at a concentration of 0.1 mg/mL and then began trending downward at higher concentrations. Lemon balm also showed inhibition of the UGT1A1 pathway. SN-38G decreased by 84 ± 8% at 0.1 mg/mL and continued to show concentration-dependent inhibition down to near 0% of control at 0.5-1 mg/mL lemon balm concentration. It also inhibited the CYP3A4 enzyme based on the measured decrease in the inactive APC and NPC metabolites which showed 23% ± 8% and a 12% ± 9% decrease, respectively, at 0.1 mg/mL lemon balm. The enzyme activity continued to trend down to near 0% of control for APC and NPC at 1 mg/mL of lemon balm.

Conclusion
Our in vitro data demonstrate that the aqueous extract of lemon balm has the potential to generate an increased amount of the active metabolite SN-38 and a reduced amount of the inactive metabolites APC, NPC, and SN-38G. Reducing SN-38G is of particular interest as SN-38G undergoes enterohepatic recycling and represents one of the dose limiting toxicities for irinotecan. The ability to biomodulate the activation of irinotecan to a more favorable distribution of active and inactive metabolites with herbal products suggests a potentially non-toxic adjuvant therapy for chemotherapeutic prodrugs which could be extended to prodrugs from other indications as well. These data also suggest that a smaller dose of irinotecan could potentially be given with concomitant dosing of aqueous lemon balm extract and possibly achieve the same therapeutic effect with the potential for fewer side effects. Additional characterization of the extract along with cellular and in vivo based data is needed for confirmation.