

Evaluation of weight loss supplements toxicity in rifampicin pre-treated HepG2 cells

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Introduction

Obesity is defined as an abnormal fat accumulation that may impair health. It is often accompanied by serious health disorders such as cardiovascular disease, diabetes, metabolic syndrome and some types of cancer. Treatment of obesity and associated conditions usually involves simultaneous use of different therapeutic approaches including weight loss supplements. The risks of adverse drug events, drug-drug interactions, and medication non-adherence rise with polypharmacy. The cytochrome P450 enzyme superfamily is predominantly responsible for the metabolism of exogenous and endogenous xenobiotics in the liver. CYP3A4 is one of the best characterized members of the family and is reported to have significant contribution to the overall metabolism due to a number of known drugs that are CYP3A4 substrates (Guttman and Kerem, 2022). Wide spectrum of active compounds act as cytochrome inducers (carbamazepine, griseofulvin, phenobarbital, phenytoin, rifampin, St. John's wort) or inhibitors (amiodarone, carvedilol, metoprolol, verapamil) which could lead to alterations of the hepatic metabolism. Rifampicin is a known potent broad inducer of drug-metabolizing enzymes including CYP3A4 (Hendriks et al., 2020). The potential of eight weight loss supplements to exert toxicity on HepG2 cells pre-treated with rifampicin used for induction of CYP3A4 activity was examined in this study.

Materials and methods

Supplements examined in this study were supplied from the market in the Republic of North Macedonia. The HepG2 cells were pre-treated with 20 μ M Rifampicin (Thermo Scientific, China) solution for 48 h. Afterwards, HepG2 cells were exposed to test solutions prepared at three concentrations respective to the minimum, the medium and the maximum daily dosage recommended for each supplement (0.05; 0.5 and 1 mg/mL, respectively), for a period of 72 h at 37 °C and atmosphere saturated with 5% carbon dioxide. Within the treatment, at predetermined time intervals of 24, 48 and 72 h, tetrazolium bromide MTT-assay (VWR Chemicals, USA) was used to determine the cell viability against the control cells treated with rifampicin only. In addition, enzyme activities of AST, ALT, LDH, γ -GT and ALP were measured using commercially available kits (BioSystems S.A., Spain).

Results and discussion

Seven of total eight weight loss supplements examined revealed concentration-dependent decrease of cell viability ($p \leq 0.05$) compared to the control. With the viability reduction, an elevation of LDH, AST, ALT and ALP were observed. Under conditions of rifampicin-induced CYP activity, herbal active compounds may be metabolized to

biologically reactive products which can contribute to the overall toxicity of the supplement (Liu et al., 2018). In this study, the most prominent changes were observed when HepG2 cells were exposed to both solutions (at a concentration of 0.5 and 1 mg/mL) prepared from the supplement containing acai berry fruits, *Garcinia cambogia* and green tea leaf extract as dominant components. Cell viability was significantly declined up to 99% compared to the control ($p \leq 0.05$) during the first 24 h and remained constantly low regardless of the exposure time. Although the enormously high LDH activity at 24 h started to decrease, it was found to be at least 7-fold increase after total exposure time of 72 h. Only γ -GT which better reflects hepatobiliary injury showed moderately increased activity. The toxicity that was observed may be driven by green tea and/or garcinia extracts that have been reported to inhibit CYP3A4 (Misaka et al., 2013; Bolla et al., 2021). This may lead to contradictory interactions at a molecular level resulting in unpredictable adverse reactions. Moreover, in specific circumstances, phenolic groups of green tea catechins are susceptible to oxidation and generate superoxide radicals and hydrogen peroxide which can further contribute to the hepatocyte damage (Yang and Pan, 2012). Other two supplements that significantly decreased the cell viability (approx. 50% of exposed cells were viable after 72 h exposure to a maximum concentration) ($p \leq 0.05$) contain green tea extracts also. It is presumed that this mechanism may be responsible for the HepG2 cell injury when exposed to higher supplements' concentration. On the contrary, a statistically significant increase in cell viability up to 30% was observed ($p \leq 0.05$) when HepG2 cells were exposed to supplement that contains nopal cladodium. Nopal has strong antioxidant activity, but its extract is not a CYP inhibitor (Jeong et al., 2018). This was evidenced with the viability increase, especially in higher concentrations and prolonged exposure. Slight to moderate increase was found for LDH and AST activities, however, the values were normalized after 72 h exposure. It is quite interesting that the viability of cells exposed to the minimum concentration of nopal was reduced during the first 24 h. This is probably due to the small concentration of nopal which may be insufficient to exhibit antioxidative effect.

Examined weight loss supplements have diverse composition of herbal compounds that have their own metabolic pathways and can differently act under conditions of induced CYP activities. This was evidenced through the significant changes of the biochemical endpoints of hepatocellular injury that were determined. The examination of the biochemical profile revealed that additional alterations to the metabolic pathways of the components of weight loss supplements, in addition to the rifampicin induction, are likely and may significantly increase toxicity.

Conclusion

The concomitant use of certain weight loss supplements with CYP-inductive compounds can lead to adverse or toxic effects in hepatocytes. This *in vitro* assessment could help for early screening of multicomponent supplements toxicity under investigated conditions. Further studies are required to explain the underlying mechanisms.

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