

# Toxicity assessment of weight loss supplements and possible interaction risk with lipid-lowering and antihypertensive drugs in HepG2 cell line

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## Introduction

Over the past few years, a significant increase in popularity and usage of weight loss supplements is evidenced. Most of these products, considered natural and safe, are not regulated by competent drug administrations. Weight loss supplements sold on the market contain herbal compounds that have been reported to cause serious health outcomes including hepatotoxicity (Cresciolo et al., 2018; Smith et al., 2016). In lack of available data consumers may be unaware that the consumption of herbal weight loss supplements may result in severe side effects. Since these products are composed of multiple ingredients or administered concomitantly with medicines they pose a risk for serious interactions. The last is a major concern as many who use herbal supplements are self-medicating and not disclosing this to health practitioners. The assessment of toxicity is an important issue yet to be studied, especially due to the concomitant use of weight loss supplements and prescribed drugs involved in the treatment of diseases related to overweight and obesity. Due to the main role of the liver in the biotransformation of xenobiotics, we have aimed to investigate the possibility for liver toxicity when multicomponent weight loss supplements are used as well as in combination with the lipid-lowering and antihypertensive drugs.

## Materials and methods

A human hepatoblastoma HepG2 cells (HepG2 cell line human, Sigma Aldrich) were used to examine toxic effects of eight weight loss supplements available at the market in Republic of North Macedonia in experiments where the concomitant use with the drugs atorvastatin and bisoprolol was simulated. The HepG2 cells were exposed to three different concentrations from each supplement: 0.05, 0.5 and 1 mg/mL (calculated following the daily dose recommendations), for a period of 72 h at 37 °C in 5% CO<sub>2</sub>. The concentration of atorvastatin and bisoprolol in the experiments simulated the average therapeutic plasma concentrations. After exposure to each supplement and with atorvastatin or bisoprolol, cell viability was determined by MTT-assay. AST, ALT, LDH,  $\gamma$ GT and ALP enzyme activity in cell culture supernatants was determined as biochemical endpoint of hepatocellular injury, following the manufacturer's protocol (BioSystems S.A., Spain) after 12, 24, 48 and 72 h of the exposure.

## Results and discussion

The viability of HepG2 cells was not significantly reduced ( $p \geq 0.05$ ) when exposed to most of the examined weight loss supplements regardless the concentration and the exposure time. The exception was observed for one multicomponent supplement containing acai berry fruit extract as the main active compound. The first 24 h of exposure to both medium and maximum concentration resulted in a significant reduction of cell survival up to 95% ( $p \leq 0.05$ ). Correspondingly, activities of LDH and aminotransferases were significantly elevated in the cell culture supernatant ( $p \leq 0.05$ ). The increased activity of the cytosolic LDH released into the medium (up to 725% compared to control) can be considered as good indicator of cytolysis. The rise in LDH activity is in conjunction with the decrease in the mitochondrial succinate dehydrogenase activity evidenced by reduced cell viability in the MTT-assay. At the initial phase of cell membrane damage more prominent increase is usually seen in ALT activity followed by rise in AST activity in severely damaged hepatocytes (Borlak et al., 2014). Subsequently, we have found a slight increase in ALT activity with the highest value of 158% of the control after 12 h-exposure to maximum concentration, probably due to much earlier cell membrane damage. However, after 24 h-exposure of cells to medium and maximum concentration AST activity was significantly increased up to 344 and 778% of the control ( $p \leq 0.05$ ), respectively. According to our knowledge, there are no data showing toxicity associated with the usage of acai berry fruit. Besides a few other compounds, the composition of this supplement includes *Garcinia cambogia* extract (active compound: hydroxycitric acid) and *Camellia sinensis* extract (active compounds: caffeine and epigallocatechins), which may be responsible for the observed effect. A case of liver transplantation has been described due to a multicomponent weight loss supplement containing hydroxycitric acid and green tea extract (Smith et al., 2016). On the contrary, other investigated multicomponent supplements that contain green tea extract or hydroxycitric acid combined with *Citrus aurantium* or ginger extract supported the cell survival during initial exposure (24 h). As a result of the multicomponent composition, different types of interactions are possibly stimulating or inhibiting cell survival. The concomitant exposure of HepG2 cells to supplement's solution and atorvastatin or bisoprolol significantly affected cell survival and in the case of certain supplement-atorvastatin combined treatment revealed an almost 50% reduction ( $p \leq 0.05$ ). The plasma level of  $\beta$ -blocker nadolol was significantly lowered after single administration of green tea extract (Abe et al.,

2018), while simvastatin pharmacokinetics in rats was changed (Misaka et al., 2013). Further, herbal compounds that inhibit CYP450 enzymes affected bioactivation of drugs which may inhibit the conversion of toxic metabolites to less toxic ones (Brewer and Chen, 2017). Since the legislation does not require investigation of interactions between herbal supplements and CYP450 enzymes, patients should be promptly advised to avoid such co-administration.

## Conclusion

The *in vitro* experiments performed in our study revealed potential hepatotoxicity related to the use of multicomponent herbal weight loss supplements. These findings should be considered as an alarm for healthcare providers as well as for patients since supplements are concomitantly used with medicines. More close regulation of weight loss supplements is an emerging issue requiring serious effort to be applied in order to protect public health.

## References

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