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Rebuttal to a Comment on Safety and Antioxidant Activity of a Pomegranate Ellagitannin-Enriched Polyphenol Dietary Supplement in Overweight Individuals with Increased Waist Size

In response to the comments by Dr. Faria raising the possibility of toxic effects of pomegranate extract supplements, it is important to emphasize that the supplement studied in our recent paper (POMx, POM Wonderful, Los Angeles, CA) contains total polyphenols (measured as gallic acid equivalents) comparable to the amounts found in a single glass of 100% pomegranate juice squeezed from whole fruit. The usual dose is one or two capsules per day corresponding to the equivalent amount of polyphenols in one or two glasses of pomegranate juice per day. This is not an excessive amount, as pomegranate juice in these amounts has been consumed traditionally with no untoward effects.

Therefore, I agree with the philosophy that supplements should not contain excessive amounts of phytochemicals and that very high concentrations of some polyphenols can be toxic, it is clear that this consideration does not apply to the specific supplement we studied.

In my view, there is no real concern at these doses of polyphenols related to hepatic cytochrome P450 activation as observed in mice. The studies of Farkas et al. (*1*), on the lack of interference of pomegranate juice in humans with midazolam as a probe for cytochrome P450 3A activity, are relevant because this enzyme is involved in the first-pass metabolism of numerous commonly used drugs. The effect of pomegranate juice (PJ) or grapefruit juice (GFJ) on CYP3A activity was studied in vitro and in healthy human volunteers. In human liver microsomes, the mean 50% inhibitory concentrations (IC_{50}) for PJ and GFJ versus CYP3A (triazolam α-hydroxylation) were 0.61 and 0.55% (v/v), respectively, without preincubation of inhibitor with microsomes. After preincubation, the IC_{50} for PJ increased to 0.97% ($P < 0.05$), whereas the IC_{50} for GFJ decreased to 0.41% ($P < 0.05$), suggesting mechanism-based inhibition by GFJ but not PJ.

Pretreatment of 13 human volunteer subjects with PJ (8 oz) did not alter the elimination half-life, volume of distribution, or clearance of intravenous midazolam at a 2 mg dose. Administration of PJ also did not affect C_{max}, total area under the curve (AUC), or clearance of oral midazolam (6 mg). However, GFJ (8 oz) increased midazolam C_{max} and AUC by factors of 1.3 and 1.5, respectively, and reduced oral clearance to 72% of control values. Thus, PJ does not alter clearance of intravenous or oral midazolam, whereas GFJ impairs clearance and elevates plasma levels of oral midazolam. This establishes that there is no intestinal or hepatic effect on CYP3A comparable to that seen with GFJ.

In vitro alterations of cytochrome P450 activity often give false-positive results that are not confirmed on in vivo testing as described in these studies. The data of Faria et al. (*2*) were obtained in mice, and the relevance to human drug−nutrient interactions has not been established. In human studies, supplementation of pomegranate juice at increasing polyphenol concentrations up to that present in two glasses of pomegranate juice per day had no adverse effects on atherogenesis (*3*). Furthermore, the alteration of metabolic enzymes in the liver by phytochemicals from fruits, vegetables, and spices is not surprising given that the liver metabolizes these substances for excretion.

Interpreting these effects as causing oxidant stress is not correct. In fact, recent studies demonstrated that POMx, as well as other pomegranate tree extracts at equivalent content to that used in the present study, significantly reduced oxidative stress and atherogenesis, with no toxic effects (*4, 5*).

I agree that in vitro effects have been shown in several studies as confirmed in the Farkas study, which also showed that these observations do not translate to clinical effects. In vitro, tropical fruits inhibit the midazolam 1′-hydroxylase activity of CYP3A. In one study, incubation with pomegranate juice led to 3.2% residual enzyme activity compared with control. In comparison, grapefruit juice resulted in 14.7% residual activity (*6*). In a similar in vitro study, Hidaka et al. (*7*) showed that incubation of pomegranate juice (5% v/v) with human liver microsomes resulted in 1.8% residual CYP3A activity for converting carbamazepine to carbamazepine 10,11-epoxide. Although these two studies indicated that pomegranate juice has inhibitory activity comparable to that of grapefruit juice, a more recent in vitro study measuring CYP3A-catalyzed midazolam 1′-hydroxylation showed that grapefruit juice had greater inhibitory potency than pomegranate juice (*8*). Despite these studies, the fact is that the study by Farkas et al. (*1*) takes precedence because it was done in humans and demonstrated the lack of a mechanism-based inhibition of CYP3A enzyme activity.

Finally, no clinically relevant interactions involving pomegranate juice and drugs metabolized by CYP3A have yet been reported. Because the effect of pomegranate juice on drug metabolism of rats may differ from that of humans, pharmacokinetic studies in human subjects must be considered before pharmacists, physicians, or nutritionists make any recommendations regarding concomitant use of pomegranate juice and CYP3A substrates. The in vitro effects of pomegranate polyphenols on CYP3A activity cannot be assumed to occur in humans at this time. The real issue is that such dietary supplements be designed following good manufacturing practices as was done with the supplement studied and that the amounts of phytochemicals contained in a supplement generally contain the types and amounts of phytochemicals in the nutritional range ingested with usual servings of fruit or fruit juice. This was clearly the case for the POMx supplement that we studied.

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