PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Systemic Exposure to Menthol Following Administration of Peppermint Oil to Paediatric Patients
AUTHORS	Kearns, Gregory; Chumpitazi, Bruno; Abdel-Rahman, Susan; Garg, Uttam; Shulman, Robert

VERSION 1 - REVIEW

REVIEWER	Bill J. Gurley University of Arkansas for Medical Sciences United States
REVIEW RETURNED	20-May-2015

GENERAL COMMENTS	This is an interesting article describing menthol disposition in paediatric patients with irritable bowel syndrome following oral administration of a delayed-release peppermint oil preparation. There were a few issues that need to be addressed before the article is acceptable for publication. The first shortcoming was one brought up by the authors and that is the paucity of subjects recruited for the study (n=6) and that sufficient data to characterize the elimination phase of the concentration-time profile was available from only 3 subjects. Although the authors attempted to utilize a statistical moment approach to account for the missing data points, the results are still questionable. Even though the study was primarily descriptive in nature, a power analysis to justify the number of subjects would have been useful. There was no independent analysis of the menthol content of the product. Given the inconsistent phytochemical content present in many nonprescription products and dietary supplements, this oversight should be addressed. The kinetics are not that of free menthol, but rather unconjugated plus conjugated menthol. The addition of beta-glucuronidase during sample preparation precluded an assessment of unconjugated menthol. A separate assessment of the kinetics of free (non-conjugated) menthol would have been useful for for understanding menthol disposition. Do the authors know to what extent menthol is sulfated? Most phytochemicals undergo significant presystemic metabolism including both glucuronidation and sulfation. Is there any indication whether conjugated menthol affects IBS? On page 11, line 15, change the word "methanol" to "menthol." On page 13, lines 38-54, the discussion focuses on menthol, but the concentration-time data reflects both conjugated and unconjugated

	menthol. Please explain how this might affect the interpretation of the results. On page 14, lines 17-22, the issue of safety regarding pulegone and menthofuran is discussed. Was the presence or absence of these compounds confirmed in the test product? This is another reason for conducting an independent analysis of the phytochemical content of the test product.

REVIEWER	Michael Rieder Department of Paediatrics Children's Hospital London Health Sciences Centre Commissioner's Road East London, Ontario Canada
REVIEW RETURNED	01-Jun-2015

GENERAL COMMENTS	This is a manuscript describing a pilot pharmacokinetic study of methanol in children following administration of peppermint oil. This is an increasingly popular but very understudied intervention in children and this is essentially the first rigorous PK study to explore this area. The experimental approach, laboratory assessment and PK analysis are conducted using standard and robust approaches. The conclusions are supported by the data. This study raises more questions than it answers, which is the mark of a highly successful pilot study. The authors appropriately note the limitations of the study and do not draw overly aggressive conclusions but rather emphasize the need for more work in this area drawing on the results of this study to inform trial design and sample size. One question that might be helpful is to know whether any of the other major components of peppermint oil (as summarized on page 5, Introduction) are likely to have any impact on the disposition or clearance of methanol and whether there are any more minor components that can impact methanol clearance. It would also be useful to state what dosage formulation of Colpermin was used (tablet or liquid). As a minor point on Page 6 the bracket before Colpermin™ should be advanced to behind it.

VERSION 1 – AUTHOR RESPONSE

Comments of Reviewer: 1

This is an interesting article describing menthol disposition in paediatric patients with irritable bowel syndrome following oral administration of a delayed-release peppermint oil preparation. There were a few issues that need to be addressed before the article is acceptable for publication.

The first shortcoming was one brought up by the authors and that is the paucity of subjects recruited for the study (n=6) and that sufficient data to characterize the elimination phase of the concentration-time profile was available from only 3 subjects. Although the authors attempted to utilize a statistical

moment approach to account for the missing data points, the results are still questionable. Even though the study was primarily descriptive in nature, a power analysis to justify the number of subjects would have been useful.

The revised manuscript now clearly states that the goal of our study was an exploratory “look” at menthol PK in a pediatric cohort following a single oral dose of peppermint oil (PMO). We now state that an a priori power analysis was not done given that the population represented a convenience sample and no comparisons (within or between-group or between studies) were made. We have also included an appropriately qualified declaration of what we feel the importance of our pilot study findings is to current knowledge and also, future studies of PMO in pediatric patients with IBS or other functional GI disorders.

There was no independent analysis of the menthol content of the product. Given the inconsistent phytochemical content present in many nonprescription products and dietary supplements, this oversight should be addressed.

The kinetics are not that of free menthol, but rather unconjugated plus conjugated menthol. The addition of beta-glucuronidase during sample preparation precluded an assessment of unconjugated menthol. A separate assessment of the kinetics of free (non-conjugated) menthol would have been useful for for understanding menthol disposition. Do the authors know to what extent menthol is sulfated? Most phytochemicals undergo significant presystemic metabolism including both glucuronidation and sulfation.

In the previous papers, it has been shown that in plasma free menthol exists only in trace amounts (below detection limits). This paper (Krzek J, Czekaj JS, Rzeszutko W. Validation of a method for simultaneous determination of menthol and methyl salicylate in pharmaceuticals by capillary gas chromatography with cool on-column injection. Acta Pol Pharm 2003;60:343-9.) reads “After ingestion of 100 mg of menthol traces of free menthol, however below LOQ, were detected in plasma at 30, 60 and 120 min after administration” In several other papers (citing the reason that in plasma menthol exists mostly in conjugation form), only total menthol was measured. Examples include:
Gelal A, Jacob P, 3rd, Yu L, Benowitz NL. Disposition kinetics and effects of menthol. Clin Pharmacol Ther 1999;66:128-35.

Mascher H, Kikuta C, Schiel H. Pharmacokinetics of menthol and carvone after administration of an enteric coated formulation containing peppermint oil and caraway oil. Arzneimittelforschung 2001;51:465-9.
It was for these reasons that we chose to evaluate the PK of total menthol in this exploratory pilot study.
Is there any indication whether conjugated menthol affects IBS?

Very small amount of menthol undergoes sulfation as suggested by the following study. “Gelal A, Jacob P, 3rd, Yu L, Benowitz NL. Disposition kinetics and effects of menthol. Clin Pharmacol Ther 1999;66:128-35”. The authors of this paper stated the following: “we did not try to measure the amount of sulfate conjugate because other researchers report only trace quantities of sulfate conjugate”. Given the apparent low degree of sulfation and the absence (to our knowledge) of any experimental evidence that a conjugated metabolite of menthol has the ability to bind and/or activate its specific receptor, we can not speculate on whether such metabolites influence the apparent effects of PMO in treating the symptoms of IBS.
On page 11, line 15, change the word "methanol" to "menthol."

This change has been made in the revised manuscript.

On page 13, lines 38-54, the discussion focuses on menthol, but the concentration-time data reflects both conjugated and unconjugated menthol. Please explain how this might affect the interpretation of the results.

The revised manuscript now clearly states that we measured total menthol. As above, the pharmacokinetic or pharmacodynamic consequences of the conjugated metabolites are not known to the authors.

On page 14, lines 17-22, the issue of safety regarding pulegone and menthofuran is discussed. Was the presence or absence of these compounds confirmed in the test product? This is another reason for conducting an independent analysis of the phytochemical content of the test product.

The section describing pulegone and menthofuran has been revised and appropriately "qualified" with regard to what is currently not known about these contaminants of PMO and/or their consequences to human health at doses of PMO used to treat IBS. It also explicitly states that we did not attempt to measure these analytes. The quality control data on Colpermin supplied to us by Tillott's Pharmaceutical did not indicate the presence of either pulegone or menthofuran in either the bulk PMO or the finished drug formulation.

Comments of Reviewer 2:

Please leave your comments for the authors below This is a manuscript describing a pilot pharmacokinetic study of methanol in children following administration of peppermint oil. This is an increasingly popular but very understudied intervention in children and this is essentially the first rigorous PK study to explore this area.

The experimental approach, laboratory assessment and PK analysis are conducted using standard and robust approaches. The conclusions are supported by the data. This study raises more questions than it answers, which is the mark of a highly successful pilot study. The authors appropriately note the limitations of the study and do not draw overly aggressive conclusions but rather emphasize the need for more work in this area drawing on the results of this study to inform trial design and sample size.

One question that might be helpful is to know whether any of the other major components of peppermint oil (as summarized on page 5, Introduction) are likely to have any impact on the disposition or clearance of methanol and whether there are any more minor components that can impact methanol clearance.

To our knowledge (including information supplied by the company who supplied the PMO formulation for the study), there is no information available in humans that would suggest / support that any of the major components of PMO would impact the "pharmacologic" effects of menthol.

It would also be useful to state what dosage formulation of Colpermin was used (tablet or liquid).

This has been accomplished in the revised manuscript.

As a minor point on Page 6 the bracket before Colpermin™ should be advanced to behind it.

This has been corrected in the revised manuscript.

VERSION 2 – REVIEW

REVIEWER	Bill J. Gurley

	University of Arkansas for Medical Sciences USA
REVIEW RETURNED	16-Jun-2015

GENERAL COMMENTS	The authors have adequately addressed my previous concerns.

REVIEWER	Michael Rieder Department of Paediatrics Western University London, Ontario Canada
REVIEW RETURNED	01-Jul-2015

GENERAL COMMENTS	The authors have in a thoughtful manner addressed the issues raised by this reviewer