

Dear Reviewer,

Thank you for the valuable review, which will improve the quality of our article and expand research perspectives on 6-MSITC and Wasabi. Below, we will address each of your comments. We have incorporated all the responses into the manuscript as a separate section titled "Discussion", highlighted in yellow.

Comments:

Although in vitro and in vivo animal studies have shown encouraging results, more clinical trials involving human participants are necessary to confirm the effectiveness and safety of 6-MSITC derived from Wasabi. Kindly provide one key aspect of the clinical and preclinical investigations of Wasabi.

Response:

Thank you for your valuable comment. Wasabi and 6-MSITC have pleiotropic mechanisms of action and exert anti-inflammatory, antioxidant, anti-atherosclerotic and anti-carcinogenic effects. Therefore, Wasabi has broad health-beneficial properties and the potential to be used to treat important global lifestyle-related health issues, including metabolic and cardiovascular diseases, neurodegeneration and malignancies. Advancing our knowledge about these plant-derived substances demands rigorous clinical research to provide reliable assessments of their efficacy and safety in humans. Progress in this field is crucial for validating existing findings and exploring novel applications of Wasabi and 6-MSITC in the prevention and treatment of entities discussed in the article.

Comments:

Highlight the necessity of conducting more comprehensive human trials.

Response:

We appreciate your insightful comment. Despite promising results of in vitro and in vivo animal studies and a few human studies, Wasabi and 6-MSITC are yet to be evaluated in clinical trials. The necessity for extensive clinical trials arises due to existing gaps in scientific knowledge, and while preclinical studies in vitro and in vivo provide insights into 6-MSITC mechanisms of action, the intricacies of human physiology and responses necessitate thorough clinical analysis.

The pleiotropic effects of Wasabi and 6-MSITC stem from their complex chemical composition, implicating potential interactions between components and requiring profound understanding. Clinical studies should encompass molecular mechanisms at the cellular level and, more importantly, the optimization of dosages, bioavailability, and pharmacokinetics.

Comments:

Discuss *potential dosages and delivery mechanisms for 6-MSITC* and provide a concise analysis of any *established or possible adverse reactions*.

Response:

Human studies with Wasabi and 6-MSITC, although limited, have shown promising results, but the variation in doses emphasizes the need for further research to determine optimal therapeutic ranges in clinical settings. For instance, administration of 9.6 mg/day 6-MSITC improved cognitive dysfunction symptoms in patients with chronic fatigue syndrome [61]. In another study, 6-MSITC at 20 mg/kg and 40 mg/kg reduced ALDH2 enzyme activity and protein expression, alleviating acetaldehyde-induced cytotoxicity in liver cancer cells [33]. In a clinical study involving 11 healthy volunteers, the consumption of 500 mg/day of Wasabi sulfinyl was well tolerated, with three adverse events in 2 out of 11 participants (18.2%), classified as unrelated to Wasabi intake [63].

The literature concerning the consumption of Wasabi or its active compound, 6-MSITC, primarily highlights its health benefits. Isolated instances of adverse reactions have been detailed in the work of Oka et al., with a documented case report specifically addressing cardiotoxicity following Wasabi consumption.

A notable incident involved the consumption of a substantial amount of Wasabi which led to Takotsubo cardiomyopathy in a 60-year-old woman. It was hypothesized that cardiomyopathy was a stress-induced reaction, resulting in heightened catecholamine levels and increased nervous system activity [104.]. There were no signs of anaphylactic symptoms, and the patient had no history of heart disease or other significant illnesses, with no mention of concurrent medications she might have been taking [105].

Finkel-Oron [105] discussed cases of anaphylaxis following Wasabi consumption, but no reports or descriptions of such incidents have been found in scientific databases like PubMed and Medline.

Moreover, 6-MSITC is currently available on the Japanese market as a supplement to enhance cognitive function. No severe side effects associated with its use have been reported, which indicates that 6-MSITC can be considered safe to use overall [63]. The diverse administration methods used in these studies, including oral administration and dietary supplementation, highlight the versatility and ease of use of 6-MSITC as a therapeutic substance [16], [52].

Comments:

Compare 6-MSITC therapy with existing therapeutic choices for each disease application.

Response:

Thank you for your thoughtful comment. The synthetic drugs used to manage metabolic and cardiovascular diseases, neurodegeneration, and malignancies have multiple diverse side effects in contrast to Wasabi and its compound 6-MSITC, which are characterised by the absence of reported side effects. Due to their multifaceted health-promoting potential, Wasabi and 6-MSITC show promise for adjuvant therapeutic applications while maintaining a high safety profile. Nevertheless, their clinical efficacy compared to standard therapy still needs to be investigated.

Comments:

Discuss constraints and difficulties in the existing research on 6-MSITC.

Response:

We appreciate your detailed comment. Analysis of existing studies on 6-MSITC reveals several limitations and challenges that may affect this molecule's comprehensive understanding and clinical utilisation. One of the main challenges is the limited availability of detailed pharmacokinetic data concerning 6-MSITC. Thus, this molecule's absorption, distribution, metabolism, and excretion processes in humans are not fully understood. Consequently, many conclusions are based on analogies to other isothiocyanates, which can lead to incomplete or inaccurate interpretations.

6-MSITC affects various molecular pathways, including the Nrf2/Keap1-ARE pathway and pathways related to glutathione metabolism and NOTCH signalling. The complexity of these mechanisms poses a challenge in accurately determining the specific biological effects of 6-MSITC and interactions between different signalling pathways. Many 6-MSITC studies have been conducted under in vitro conditions which may only partially reflect its actions in living organisms. Although in vitro results provide valuable insights into potential mechanisms of action, further in vivo studies are necessary to confirm these observations and understand how 6-MSITC behaves in more complex biological systems.

While studies suggest potential health benefits and overall safety of 6-MSITC, there is limited data on its long-term toxicity and side effects. Without accurate toxicological data, assessing the safety of long-term use of 6-MSITC is challenging and remains a question of the future. Moreover, variations in response to 6-MSITC may arise from individual genetic and biological variability. For instance, differences in the expression of enzymes metabolizing 6-MSITC can affect its efficacy and safety in different populations, so studies addressing these variabilities are needed to develop personalized approaches to therapy.

Developing precise analytical methods to measure the concentration of 6-MSITC and its metabolites in various biological matrices is crucial. Standardizations of these methods is necessary to ensure consistency and comparability of results across different research teams.

Therefore, understanding the potential of 6-MSITC in medicine requires further research that addresses these challenges. Future efforts should focus on expanding

pharmacokinetic and toxicological studies, conducting more in vivo research to validate in vitro findings, developing personalized therapeutic strategies considering genetic variability, and ultimately standardizing analytical methods and research protocols.

Comments:

Future Directions: The text would be improved by including a more comprehensive analysis of the potential future paths for 6-MSITC research. Providing precise guidelines for the design and execution of human clinical trials would offer useful direction to researchers in the field.

Response:

Providing precise guidelines for the design and execution of human clinical trials seems to be beyond the scope of this article. We limited ourselves to underline key areas requiring more thorough investigation.

We are pleased to attach the revised manuscript. The updated sections, in accordance with the Reviewer's suggestions, have been highlighted in yellow for your convenience.

Thank you for your attention and consideration.

Sincerely,
Joanna Bartkowiak-Wieczorek