Peer Review File

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Comment 1:
Significance: “With the notable pharmacological activity crocin in saffron has been investigating widely resulting ... in vivo, in vitro and in clinical trial” (lines 14-17). Why did the authors choose to review "in vitro" rather than “in clinical trial” or “in vivo” studies with more practical applications?
Reply 1: The authors selected in vitro investigation of crocin to solve the mechanism of neuroprotective activity of crocin in this review. The investigation using PC-12 cells can cover wide range of function. The results obtained from PC-12 cells can indicate neuroprotective activity, but not for learning/memory phenomenon and dementia, in other word the possibility can be suggested by the in vitro experiments. Therefore, the authors review step by step such as in vivo to clinical trial as series of multifunctional saffron.
Changes in the text: “However, the mechanisms of neuroprotective, learning/memory promoting and anti-dementia activities for crocin are not exactly clear yet.” was inserted in Background.

Comment 2:
Innovativeness: Given that there are many similar reviews in this field (PMID: 35198096, 33260389, 27651258) and the HIGH overlapped results and conclusion in the similarity report (sources 1~5), what does this review add to existing knowledge? How does this review differ from previous reviews? Please clearly state this.
Reply 2: The author added 2 sections in this revie such as “1. Preparation of anti-crocin monoclonal antibody and immunostaining of PC-12 cells” The MAb confirmed the incorporation of crocin in neuronal cells and can be used for the distribution of crocin in the cells. Furthermore, since the newly prepared MAb has wide cross-reactivity against crocetin glycosides, it can be applied for metabolic study and for pharmacologically active mechanism of crocin in the central nervous system. Such suggested documents were inserted in the new section.
The other new section “5. Proteins in central nervous system” was prepared. Regarding neuroprotection many proteins are associated. Among them the author selected several proteins mainly such as CaMKII because this protein is closely related to LTP in vitro. However, no change of CaMKII level was occurred by the injection of crocin in vivo. Such difference between in vitro and in vivo were incorported in the section.
Changes in the text: New two sections “1, Preparation of anti-crocin monoclonal antibody and immunostaining of PC-12 cells” and “5. Proteins in central nervous system” were incorporated.

Comment 3:
Rationality: "Saffron is a well-known and safe food as oral administration of saffron extract at concentrations of up to 5 g/kg, was nontoxic in human (28). Therefore, the neuroprotective activities of saffron will be incorporated in the current study" (lines 112-114). After reading reference 28, there are three questions:
(1) The editorial is not the source literature for this finding, it should be this one by Ramadan et al.: "Ramadan A , Soliman G , Mahmoud S S , et al. Evaluation of the safety and antioxidant activities of Crocus sativus and Propolis ethanolic extracts[J]. Journal of Saudi Chemical Society, 2012, 16(1)".
(2) 5g/kg is the animal dose, not the human dose.
(3) Although the editorial concludes that "at the same time should not deter the therapeutic utilization of this medicinal herb", several studies mentioned in the text also mention the toxic and adverse effects of saffron, and it is suggested that the authors rewrite it to reflect the rationality of choosing saffron.
Reply 3: Regarding the editorial suggestions the author newly selected and incorporated three papers related to safety of saffron and crocin in healthy volunteers and AD patients.
Changes in the text: [Saffron is a well-known and safe food as oral administration of saffron extract at concentrations of up to 5 g/kg, was nontoxic in human (27).] was removed. The following sentence was inserted in line 125. [In the case of health volunteers saffron (200 and 400 mg tablets) are safe drug on coagulation system under a double-blind, placebo-controlled study for 1 week (Ayatollahi et al. 2014). When old AD patients were administered saffron extractives (30 mg) or donepezil (10 mg) as a positive control under a double-blinded/phase II study, the effect of saffron extract was evaluated to be the same with donepezil for mild to moderate AD patients. The major adverse effect of saffron, vomiting was lower than that of donepezil (Farokhnia et al, 2014). Volunteers received crocin tablet (20 mg) or placebo resulted that the crocin tablet showed no major side effect except decreases of amylase, white blood cells and partial thromboplastin time (Mohamadpour et al. 2013).].

Comment 4:
Methods: There is no Methods section provided. Detailed literature search information can help assess whether the search is comprehensive and up-to-date.
Reply 4: Search engine, survey term and language were confirmed and the obtained data were analyzed and selected to cite as references.
Change in the text: “A narrative review of data published on the pharmacological value of saffron and its constituents were surveyed and collected references gathering from the Google search engine from 1980s until April 1st 2022 and publications in English. The suitable data of investigations have been compared, analyzed and reorganized resulting in newly assembled clarification.” was inserted in Methods section. Furthermore, Table 1 and 2 were added in in Method in Introduction section.

Comment 5:

Enlightenment: After reviewing the in vitro neuroprotective effects of saffron, could the authors consider giving their personal views and suggestions on the current status, problems, and future directions of this field? In addition, we suggest that the authors could explain how these in vitro studies could be better applied to clinical trials, whether there are obstacles and whether there are feasible solutions. This may help readers to think about these issues and gain new ideas.

Reply 5: The author prepared three reviews such as “The effect of crocin on neuroprotective activity in vitro, a narrative review”, “Learning and memory activities of saffron and its constituent crocin, a narrative review” and “Pharmacological activity of saffron and its component on dementia, a narrative review.” In vitro, in vivo and clinical trial have important feature individually. In vitro results can cover the wide range of mechanism and suggestion. When a researcher makes planning of clinical research, in vivo results derived from mainly in vitro research can help for decision resulting that three categories of research are necessary. This is the reason why the author prepared three reviews step by step as indicated above in order to give the best choice for readers.

Comment 6:

Introduction:

(1) I'm curious if the different extraction processes (alcoholic extracts, aqueous extracts) affect the active components of saffron and their content? Does this have any effect on neuroprotective activity?

Reply: The extraction solvent affects the concentration of crocin, major active constituent in the extract. So that individual references showed the extractive solvent. Usually the ethanol extract is used for investigation because evaporation of solvent is easier than water. The concentration of crocin in aquas extractives and ethanol extractives is not so different because crocin which possess 4 glucoses in a molecule can be solved both in ethanol and water. Since crocin is unstable, the quality control of saffron and/or crocin is necessary. Therefore, anti-crocin monoclonal antibody which can analyze the concentration of crocin quickly and accurately was prepared in this review.

(2) line 91-92: "blood disorder (5), anti-cancer(6-8),anti-oxidant activity (9, 10), memory impairment (11-16)". For consistency, would it be better to replace"anti-cancer, antioxidant" with "cancer, oxidative aging"

Reply: Since anti-cancer activity and anti-oxidant activity are used widely, the author wishes to use them in this review.

(3) Also, please specify the objective in the end of the introduction.

Reply: Background and Objective, Method, Key Content and Findings and conclusion were prepared separately in Abstract.

Comment 7:

Given that the review focuses on studies related to PC-12 cells, we suggest the authors briefly introduce it (e.g., "PC12 cells are a commonly used neural cell line, ...")

Reply 7: “The wide field data related to the neuronal investigations has been accumulated by the rat pheochromocytoma (PC-12) cells easily differentiated into neuron-like cells. Therefore, the neuroprotective activity of crocin using PC-12 cells is reviewed.” was inserted.

Comment 8:
Lines 126-224: "Irma et al. found that…". We suggest the authors could present their argument first and then provide its supporting details, which would help readers to read with questions in mind.
Reply 8: “Irma et al found that…” The sentence was changed. As suggested “Rat pheochromocytoma (PC-12) cells have been used widely for the neuronal investigation because it is easily differentiated into neuron-like cells. Furthermore, the clear cell death is induced by serum and/or glucose deprivation in culture medium.” was added in the first sentence.