Author’s response to reviews

Title: The use of Euphorbia hirta L. (Euphorbiaceae) in diarrhea and constipation involves calcium antagonism and cholinergic mechanisms

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Response to Comments

Editor's comments:

1. At present, we do not feel that there is sufficient evidence presented in your Background section to justify the testing of Euphorbia hirta in two animal models. We would therefore ask you to expand this section to include as much referenced evidence as possible to explain why you would expect this treatment to have an effect in this model. This evidence should come from previous in vitro or animal work. Please note that we are unable to accept traditional medical use as sufficient justification for animal studies.

Response:

Thank you for giving us this opportunity to further improve

In support of the dual medicinal utility of E. hirta in opposing gut disorders like diarrhea and constipation, previous reports have shown unclear findings like, Tona et al. [20] has revealed non-specific antispasmodic action of E. hirta in the in vitro and Hore et al. [21] demonstrated only gut inhibitory effects of E. hirta in naïve rats and castor oil administered mice. While, Galvez et al. [22] reported its gastrointestinal transit delaying potential in only castor oil administered animals. On the other hand, Kamgang et al. [23] showed its gut stimulant effects in the in vitro only and antidiarrheal activity in the in vivo only. Keeping in view the folk use of E. hirta in diarrhea and constipation, and to further explore the paucity in existing literature whether it possesses both laxative and antidiarrheal effects in the in vivo and/or in the in vitro, the primary objective of this study was to determine the antidiarrheal and laxative efficacy of E. hirta and the possible pharmacological basis of the identified effects. While the secondary objective
was to estimate the distribution and comparative efficacy of gut stimulant and relaxant constituents in polarity-driven fractions of E. hirta.

Related response has also been incorporated in the revised version of the manuscript in red colored text as detailed above

2. Please clarify your euthanasia/sacrifice methods, including whether animals were anaesthetised and/or unconscious, injection dosages if applicable, methods used and rationale etc.

Response:

Thank you for giving us this opportunity to further improve

We have added related detail as

In-vitro studies

Preparation of isolated rabbit jejunum segments

The healthy adult rabbits (6–7 months old) were randomly selected for the study and were anaesthetized using thiopental sodium injection at dose range of 70-100 mg/kg, once deeply anaesthetized, cervical dislocation was performed by a blow on the back of the head and subsequently dissection was carried out by an incision on abdominal side. The abdomen was cut open using sharp edged blade and the jejunum was isolated and immersed into Tyrode’s solution in petri dish aerated with carbagen (95% O2 and 5% CO2).

Preparation of rat ileum

The healthy adult Sprague–Dawley rats (8-12 weeks old) were randomly selected and starved for 12-16 h. Rats were anesthetized using isoflurane (2-5 % v/w) through inhalation in a closed chamber until achievement of deep anesthesia. Once animals were deeply anaesthetized, cervical dislocation was performed with the help of rod placed on the neck of animal followed by pulling the tail of the animal opposite to the neck, subsequently dissection was carried out by an incision on the abdominal cavity. The abdomen was cut open using sharp edged blade and the rat ileum was isolated.

Related response has also been incorporated in the revised version of the manuscript in red colored text as detailed above

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- Ethics approval and consent to participate
- Consent to publish
- Availability of data and materials
- Competing interests
- Funding
- Authors' Contributions
- Acknowledgements
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Response

We are sorry, Authors' Information was missing, we have provided accordingly in revised manuscript

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2. We have also provided the data as supplementary files which was not shown in the manuscript earlier

We feel that with your very valuable suggestions and their addition in revised manuscript, the revised manuscript has been improved.

Regards,

Hassan