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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Author’s response to reviews:

Response:

Editor Comments:

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

In methodology section, we have revised in red colored text as advised and incorporated in the revised manuscript;

In-vitro studies

Preparation of isolated rabbit jejunum segments

The healthy adult rabbits (6–7 months old) were selected randomly for the study and anaesthetized using intraperitoneal injection of thiopental sodium at dose range of 70-100 mg/kg. Sodium thiopental was purchased from Care Pharmacy, Faisalabad, Pakistan. Once rabbit was anaesthetized and confirmed by absence of touch and corneal reflexes, thoracotomy was performed followed by cardiac puncture to euthanize the animal. The abdomen was cut open using sharp edged blade and the jejunum was isolated and immersed in Tyrode’s solution in petri dish aerated with carbogen (95% O2 and 5% CO2). The mesenteries were carefully removed. Individual segments of jejunum (2-3 cm) were hanged in 10 mL tissue organ baths containing Tyrode’s solution maintained at 37°C and aerated by carbogen. Tyrode’s solution contained KCl; 2.68, NaCl; 139.9, MgCl2; 1.05, NaHCO3; 11.90, NaH2PO4; 0.42, CaCl2; 1.8 and
Glucose; 5.55 in mM with a pH 7.4. Administered dose of thiopental sodium did not affect normal activity pattern of isolated tissue in tissue organ bath.

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 fully anesthetized. Isoflurane was purchased from Care Pharmacy, Faisalabad, Pakistan. Once animals were fully anaesthetized and confirmed by absence of touch and corneal reflexes, thoracotomy was performed followed by cardiac puncture to euthanize the animals. The abdomen was cut open using sharp edged blade and the ileum was isolated. Individual ileum segments of 2-3 cm were suspended in 10 mL tissue organ baths containing Tyrode’s solution, maintained at 37°C and aerated by carbogen. A preload of 0.7-1.0 g was applied to individual tissue. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with repeated administration of acetylcholine (Ach, 0.3 µM) at every 3-5 min interval, until similar responses were achieved. Exposed dose of isoflurane did not alter the response of Ach on isolated tissue in tissue organ bath.

We have also got our manuscript reviewed from our colleague, Department of English, Government College University, Faisalabad, Pakistan

We hope that in current form our manuscript has been improved.

Regards,

Dr. Malik Hassan Mehmood