Author's response to reviews

Title: Antinociceptive, muscle relaxant and sedative activities of gold nanoparticles generated by methanol extract of Euphorbia milii

Authors:

Nazar Ul Islam (islanaz@yahoo.com)
Ibrahim Khan (ibrahimchemist87@yahoo.com)
Abdur Rauf (mashalics@yahoo.com)
Naveed Muhammad (drnaveedrph@gmail.com)
Muhammad Shahid (shahidsalim_2002@hotmail.com)
Mohammad R Shah (raza_shahm@yahoo.com)

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Author's response to reviews: see over
The Editor-in-Chief,

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Subject: Submission of revised manuscript for publication

Dear Sir,

Thank you for your useful comments and suggestions related to our manuscript. We have modified the manuscript accordingly, and point-by-point corrections are listed below:

1) Experimental research on vertebrates or any regulated invertebrates must comply with institutional, national, or international guidelines and where available should have been approved by an appropriate ethics committee. A statement detailing compliance with guidelines and/or ethical approval must be included in the manuscript. For studies involving client-owned animals, authors must state in the manuscript that informed client consent was obtained and adhere to a high standard (best practice) of veterinary care. Authors are encouraged to conform to the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines (http://www.nc3rs.org.uk/page.asp?id=1357) for reporting animal studies.

Answer: The experiment protocols on animals were approved by the Ethical Committee of the Department of Pharmacy, University of Peshawar, Peshawar, Pakistan. Moreover the Ethical Committee of the Department of Pharmacy, University of Peshawar strictly follows the UK Animals (Scientific Procedures) Act 1986 guidelines.

The Animals section of the Materials and Methods has been changed to:

BALB/c mice of either sex weighing 25-30 gm were purchased from the Pharmacology section of the Department of Pharmacy, University of Peshawar, Peshawar, Pakistan. The animals were maintained in a 12 h light/dark cycle at 22 ± 2 °C. Access to food and water was *ad libitum*. Experiments on animals were performed between 9:00 am and 3:00 pm. The experiment protocols were approved by the Ethical Committee of the Department of Pharmacy, University of Peshawar, Peshawar, Pakistan.
2) At present, we do not feel that there is sufficient evidence presented in your Background section to justify the testing of the neuropharmacological effects of *Eurphorbia milii* in an animal model. 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.

**Answer:** The background section of the manuscript has been revised as suggested by the editor. The Background has been changed to:

Herbal medicines have been widely recognized by physicians and patients for their better therapeutic value and fewer adverse effects as compared to modern medicines [1]. Herbal medicines require unnecessarily high systemic administration due to their non-specific biodistribution [2]. Drug delivery systems within the nanometer size regime can be developed to alter both pharmacological and therapeutic effects of drug molecules [3]. Phytotherapeutics need a scientific approach to deliver the components in a novel manner by increasing bioavailability and reducing toxicity, which can be achieved by designing nano drug delivery systems for herbal constituents. Applications of nanotechnology to herbal drugs may lead to the development of nano-herbal products which will open a new era of herbal drug discovery [1].

The family Euphorbiaceae consists of 2000 species. The genus *Euphorbia* is the largest genus in medicinal plant kingdom which is widely distributed in China and Pakistan. Some species of the genus *Euphorbia* have been traditionally used for the treatment of skin diseases, gonorrhea, migraine, and intestinal parasites and as wart cures [4]. The genus *Euphorbia* has been studied widely for its antiproliferative [5-6], cytotoxicity [7-8], tumor promoting [9-10], antimicrobial [11-12], antidiarrheal [13], antidiabetic [14], molluscicidal [15-16], urease inhibition [17], angiotensin converting enzyme inhibition [14], antipyretic [18-19] and analgesic [20-21] activities. The biological research on *Euphorbia* species has been supported by the use of some plants in traditional medicines or revealed the new activities on modern pharmacological levels [22]. The sedative, anxiolytic, analgesic, antipyretic and anti-inflammatory properties have been reported for
E. decipen [21], E. resinifera [23-24], E. fischeriana [23], E. royleana [25], E. heterophylla [26], E. hirta [18] and E. milii [27].

Euphorbia milii commonly known as the crown of Thorns is used for ornamental purposes and have not been reported in folk therapy in Pakistan; however, in Nepal the latex is used for treating strains [28], while in China it is used for the treatment of hepatitis and abdominal edema [29]. The undiluted latex of E. milii was found to be irritant to mammalian eyes and skin [30]. Phytochemical studies of E. milii revealed the presence of β-sitosterol, cycloartenol, β-amyrin acetate, lupeol, euphol, flavonoids and triterpenes [31-32]. Some of their diterpene esters of ingenol are potent skin irritants but, in contrast to other closely-related ingenol and phorbol derivatives they have no tumor promoting activity [33]. Milliamines isolated from E. milii latex exhibited potent molluscidal activity [16]. In previous study, the crude methanol extract of E. milii showed significant analgesic activity comparable to that of diclofenac sodium [27].

The present work demonstrates green synthesis of gold nanoparticles using a methanol extract of E. milii (Au-EM) with characterization, metals sensing ability and their screening for antinociceptive, muscle relaxant and sedative activities.

3) The title of the manuscript has been changed to “Antinociceptive, muscle relaxant and sedative activities of gold nanoparticles generated by methanol extract of Euphorbia milii”.

4) The background section of the abstract has been revised.

The manuscript has been resubmitted to your journal. We look forward to your positive response.

Best Regards,

Prof. Dr. Nazar Ul Islam

Department of Pharmacy

Sarhad University of Science and Information Technology

Peshawar, KPK, Pakistan