e-ISSN: 2320-4230, p-ISSN: 2961-6085

Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 11, Issue 03, May-June: 2023, 52-55

Pharmacological Effects of Methanolic Extracts of Mormordica Charantia in Vincristine Induced Peripheral Neuropathy

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Received: 17-04-2023 / Revised: 16-05-2023 / Accepted: 19-06-2023

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Conflict of interest: No conflict of interest.

Abstract:

To conclude, both methanolic extract of *Mormordica charantia roots* as well as ethanolic extract of *Moringa oleifera* leaves showed potential neuroprotective activity as they have effective inducible NOS (iNOS) and neuronal NOS (nNOS) inhibiting property, antioxidative and anti-inflammatory activity. The saponins obtained from methanolic extract of *Mormordica charantia roots* showed antinociceptive activity and amelioration of both nerve conduction velocity (NCV) and histopathological changes (sciaticnerve) after vincristine induced alteration.

Keywords: Mormordica charantia Methanolic extracts, Vincristine, peripheral neuropathy

INTRODUCTION

Momordica charantia, commonly known as bitter melon, is traditionally used in various cultures for its medicinal properties. Recent research highlights its potential benefits in managing diabetes and protecting liver health. Peripheral neuropathy refers to a condition resulting from damage to the peripheral nerves, which are the nerves outside the brain and spinal cord. It affects the transmission of signals between the central nervous system and the rest of the body[1]. This work was aimed to determine Pharmacological effects of methanolic extracts of Mormordica charantia In vincristine induced peripheral neuropathy

Materials and method:

The Chemical used in the study include Vincristine sulphate purchased from the market with brand name Cytocristin1mg/1ml(Cipla) and Methylcobalamin injection solution with brand name Methylcobal (Eisai) manufactured by Wockhardt limited. Methanol fromFisher Scientific, ethanol from SD Fine Chem. Chemicals such as Molisch's reagent, Sulphuric acid, Ferric chloride, Sodium hydroxide, Hydrochloric acid, Carbonate buffer, DMSO, Sodium carbonate buffer, Trichloroacetic acid (TCA), Chloroform, NaCl from Qualigens (Thermo Fisher Scientific Chemicals, India). Mayer's reagent, L-ascorbic acid, Thiobarbituric acid (TBA), Griess reagent from LOBA Chemie Pvt Ltd. Mumbai. Glacial acetic acid from Fisher Scientific. Copper sulphate,Acetone,Tween20,

Phosphatebuffer,Acetic anhydride, Ammonia solution from Merk, India All other Chemical used where of Analytical Reagent Grade(AR Grade)

Extraction: For seven days, the roots of Mormordica charantia were dried in the shade at room temperature. The dried roots were pulverised and sieved (coarse 10/40). Thepowder was used to make a methanolic extract of Mormordica charantia. In a Soxhlet extractor, the dry powder was extracted with methanol using batches of 250g of the dry powder every time. The initial extract was filtered and allowed to evaporate so as to obtain a concentrate on a rotary shaker at 55°C and subsequently dried under vacuum[2].

Experimental animals:

Wistar rats Male (8-10 weeks old) weighing 150–200g and *Albino mice* Female (8-10weeks old) weighing 20-25gm were used for the experiment. They were acclimatized for one week prior to experiment. Animals were caged in fully ventilated room,were maintained in 12:12 h light and dark cycle and were housed at temperature of 25 ± 2°C.They had free access to a standard chow diet and water *ad libitum*. All the experiments conducted on the animals were in accordance with the standards set for the use of the laboratory animal and the experimental protocols were duly approved by the IAEC of Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra (ReferenceNumber:CPCSEA/IAEC/48/2019-20/164).

Acute toxicity study:

The acute oral toxicity study was performed according to the OECD guidelines No.425

the animals were fasted overnight and limit test was performed as follows[3];

The limit test was carried out first at 2000 mg/kg body weight for one animal and if animal dies, main test is performed. If the animal survives two more animals are dosed, if both survives the test is terminated, the main test is performed with an initial dose of 175 mg/kg body weight. Following sequence was followed 175, 550, 1750 and 5000mg/kg body weights.

First one animal is dosed with 175mg/kg body weight. If animal dies a much lower dose is tested. If animalsurvives, then two more animals are dosed after 48 h observation of the first animal. If animals survive then the main test should be terminated. If animal dies, two more animals are dosed and observed. The dosing is stopped when one of the following criteriais met:

1. Three consecutive animals survive at the upper bound
2. Five reversal soccurinany six consecutive animals tested.
3. At least four animal shave followed the first reversal and the specified likelihood ratio sex ceed the critical value.

Preparation of Dose:A dose of 1/10th and 1/20th of 2000mg/kg were considered to be the high dose and low dose respectively; doses were prepared by dissolving in mili Qwater.

Pharmacological effects of methanolic extracts of Mormordica charantia In vincristine induced peripheral neuropathy(VIPN)

Peripheral neuropathy was induced in rats by intraperitoneal injection of vincristine (100µg/kg/day) body weight dissolved in saline (0.1 ml/kg/day) to induced peripheral neuropathy . All the animals were allowed free access to tap water and normal chow pellet diet and maintained at room temperature in polyethylene cages.

Group classification:

Rats were induced vincristine (100µg/kg/day) for14consecutive days later on administered standard and test compounds for 21 days. 30 healthy rats were divided into following groups, where N=6 Animals in each group.

Group 1: Administered only vehicle (saline solution) serves as normal control
Group2: Administered vincristine (100µg/kg/dayi.p.),serves as neuropathy control
Group 3: Neuropathy animals administered reference standard, Methylcobalamin (50µg/kgi.p.)
Group4: Neuropathy animals were treated with Low dose (100mg/kg/b.w.p.o.) of methanolic extracts of Mormordica charantia, dose obtained from acute toxicity
Group5: Neuropathy animals were treated with High dose(200mg/kg/b.w. p.o) of Mormordica charantia, dose obtained from acute toxicity
After the end of experiment, the following parameters were evaluated;

Hot plate Method:

In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55°C) and there action of animals such as paw licking or jump response is taken as the end point. Normally animals show response in 6-8sec.Acut off period of 15sec in observed to avoid damage to the paws. Prior to any treatment, the animals were allowed to familiarize with the test procedure and apparatus, and baseline values were obtained[4].

Results and discussion

Mortality was not seen in the acute toxicity study up to a dose of 2000 mg/kg for methanolic extract of *Mormordica charantia*roots and a dose of 1/20th and 1/10thof 5000 mg/kg (100 mg/kg and 200 mg/kg) were considered as low and high dose for pharmacologic screening. The drugs were prepared by dissolving in miliQ water and further studies were carried out.

Hot plate Method

The pain sensation effects of VIPN in rats using hot plate is shown in Figure 1, the treated neuropathy control Wistar rats displayed a significant increase ($p<0.001$) in hot plate response time in comparison to the normal control. Where as the group receiving methanolic extracts of Mormordica charantia higher and lower dose displayed a significant change in hot plate response time $p<0.001$, $p<0.01$ respectively, when compared with standard, Methylcobalamin control.

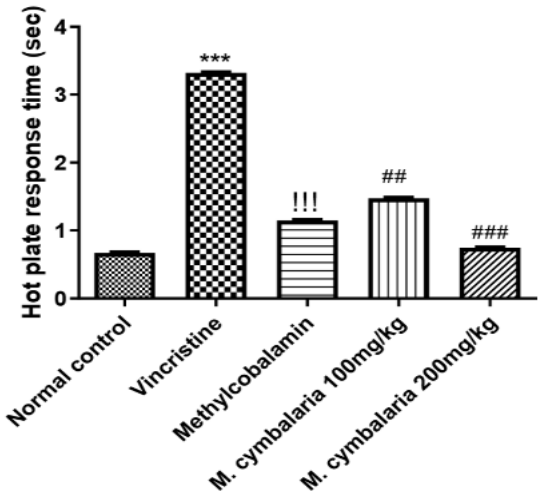

Figure 1: Pain-Sensation Effect of VPN In rats using Hot-Plate Method *Values are expressed as Mean±S.E.M (n=6).*** *p<0.001 when compared with normal control*
!!!p<0.001 when compared with disease control, ###p< 0.001, ##p< 0.01 when compared with Standard, Methylcobalamin control.

Conclusion:

The results presented here demonstrated beneficial effects of ethanolic extract of *Moringa oleifera* leaves on VIPN. The ethanolic extract of *Moringa oleifera* was subjected to acute oral toxicity studies and was found to be safe up to 5000mg/kg body weight. Ethanolic extracts of *Moringa oleifera* were studied at two dose levels i.e., 250mg/kg and 500mg/kg. The higher dose500 mg/kg dose was found to be better to the 250 mg/kg dose in terms of behavioural (thermal hyperalgesia i.e.,hot plate) alteration along with nerve conduction velocity test (NCV) and histopathological change (sciatic nerve) evaluations.

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