The objective of this study was to investigate the acute toxicity and potential activity of methanolic extract of Hyoscyamus muticus (Me-HM) to assess nociception in mice. The acute toxicity was studied in both oral and intraperitoneal route. LD50 was determined using Probit method and the effect of extract against nociceptive was studied by thermal stimulus (hot plate) and injection of chemical substances such as formalin (formalin test) and acetic acid (writhing test). Morphine was used as positive drug in hotplate test and acetylsalicylic acid was used in formalin and writhing test. The antinociceptive activity was determined by observed increase of latency time in hotplate test, decrease of abdominal constriction in writhing test and decrease of stretching in formalin test. The LD50 of intraperitonial administration of Me-HM was 1000±42, 89 mg/kg-1. Our extract produced a significant (P≤0.001) and dose dependent increase of latency time in hotplate test. The optimal effects were observed after 90 mins of oral administration of both doses. In the formalin test, the both doses reduce significantly (P≤0.001) the effect produced by intraplantar injection of formalin with maximum inhibition recorded in neurogenic phase with 49.36% and 42.67% successively for 100 and 50 mg/kg of Me-HM. Morphine and acetylsalicylic acid produced desired anti-nociceptive activity in tests used in this study. The antinociceptive effect of H. muticus extract can be explained probably by binding of scopolamine and hyoscyamine from extract to muscarinic receptors and 5-HT3 involved in pain pathways. It was concluded that Me-HM shows a remarkable antinociceptive activity in thermal and chemical model of nociception in mice.