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The protective effect of bee venom against verapamil embryotoxicity during prenatal liver and kidney development of mice *Mus musculus*

Amin A. Seleem

Zoology Dept. Faculty of Science, Sohag University, Egypt

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KEYWORDS

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Abstract Verapamil is a calcium channel blocker that has been widely used in the treatment of cardiovascular abnormalities, hypertension and angina pectoris. The present study investigates the effect of bee venom against verapamil embryotoxicity, bee venom (BV) is characterized with anticancer, anti-inflammatory, anti-rheumatoid, pain-relieving and neuroprotective agents. The current study was carried out on 70 pregnant female mice which were divided into two main groups, the first main group divided into three subgroups, control, treated with single and twice dose daily of verapamil (40 mg/kg) that was treated from zero day of gestation to scarification of females at E10. The second main group that was treated from the seventh day of gestation was divided into four subgroups, control, treated with single dose daily of verapamil (40 mg/kg), injected with bee venom (150 μg/kg/BW) and treated with verapamil combined with bee venom, the females were sacrificed at E14 and E17. The results of this study showed that verapamil treated groups once or twice daily in the first main experiment showed abortion and resorption of uteri embryos. In the second main experiment, developing liver and kidney at E14 and E17 in verapamil treated group showed abnormal architecture of histological picture and alterations of immunohistochemical expression of heat shock protein and BAK that were associated with ultrastructure abnormalities at E17. Bee venom treated group showed the similar structure as control, verapamil combined with bee venom treated group exhibited amelioration against verapamil embryotoxicity. In conclusion, bee venom could be considered as a therapeutic agent and it has a curative effect against the toxicity of verapamil during development of liver and kidney.

Introduction

Calcium channel blockers (CCBs) or calcium antagonists are among the most widely used drugs in cardiovascular medicine and regulation of blood pressure in hypertensive patients (Girvin and Johnston, 2004; Lipsitz et al., 2005; Pavlovic et al., 2004). Verapamil is an L-type voltage-dependent calcium channel blocker, belongs to the phenylalkylamine group, and has been used in the treatment of angina pectoris, cardiovascular abnormalities, hypertension, cardiac arrhythmia and coronary vasospasm (Kumar and Hall, 2003; Grossman and

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Messerli, 2004; Hirota et al., 2004; Wu et al., 2004). Verapamil given either *in vivo* or *in vitro* has been shown to inhibit calcium uptake (Goligorsky et al., 1985) and any alterations in calcium homeostasis can be quite toxic (Choi, 1992) where the concentration of cytosolic calcium is a key factor for control of many diverse cellular and pathophysiological functions. Calcium channel blockers have been shown in animal experiments to be able to induce teratogenic effects and increase the incidence of embryolethality (Fukunishi et al., 1980; Cabov and Palka, 1984; Robert et al., 2011). Verapamil, is a classic chemosensitizer which can enhance the antitumor effect in several cancer cells including neuroblastoma, lung and colorectal cancer (Ikeda et al., 1987; Wang et al., 2010; Koski et al., 2012).

Bee venom (BV) is the venom that bees store within their venom sacs for self-defense which is also known as apitoxin, it is extracted from honeybees and is commonly used in Oriental medicine to relieve pain and treat inflammatory diseases. Bee venom is known to be a very complex mixture of at least 18 active components including active peptides, melittin (a major component of BV), apamin, adolapin (Kwon et al., 2005) and amines that contain histamine, catecholamines, polyamines, melittin, and phospholipase A2 (Oršolić, 2012). Melittin is the principal component of BV and constitutes approximately 50% of its dry weight (Vogel, 1981). Bee venom is introduced natural anticancer agents (Oršolić et al., 2003; Gajski and Garaj-Vrhovac, 2011), Anti-mutagenic, (Varanda et al., 1999), anti-inflammatory (Nam et al., 2003), anti-nociceptive, (Kim et al., 2003), anti-rheumatoid arthritis (Kwon et al., 2005), pain-relieving (Kwon et al., 2001), neuroprotective (Alvarez-Fischer et al., 2013) and immune modulatory activity (Nam et al., 2005). The aim of the present study was to examine the ameliorative effect of bee venom against embryotoxicity of calcium channel blockers (verapamil) during development of liver and kidney at prenatal stages E14 and E17.

Material and methods

Chemicals

Verapamil was obtained from Sigma–Aldrich Company USA. Bee venom of *Apis mellifera* was obtained from Faculty of Agriculture, Assuit University.

Animals and experimental design

Adult female and male mice *Mus musculus* (weighting 25–30 g) were used. The animals were obtained from a closed random bred colony at Faculty of Science, Sohag University, Egypt. The mice were maintained on food and water ad libitum and housed in groups in isolated cages. The animals were acclimatized for 2 weeks prior to usage. A male and two females were placed in one cage and left overnight. The presence of the vaginal plug, next morning was considered as fertilization indicator, and was considered zero day of pregnancy. Seventy pregnant female were divided into two main groups, the first main group (primary experiment) that was treated from zero day of gestation to prenatal E10 was divided into three subgroups (10 each), control, treated with single dose daily and twice dose daily in the morning and in the evening of verapamil (40 mg/kg). The second main group (essential experiment) was designed after the results of the primary experiment. The studied pregnant females of the second main groups were treated from the 7th day of pregnancy to sacrification at prenatal stages E14 and E17 that were divided into four subgroups (10 each), control, treated with single dose daily of verapamil (40 mg/kg), treated with bee venom subcutaneously injected (150 μg/kg/BW) (Alvarez-Fischer et al., 2013) and treated with verapamil single dose combined with bee venom subcutaneously injected.

Histological examination

The whole embryos at prenatal stages E14 and E17 of studied groups were fixated in carnoy's fixative, dehydrated in ethyl alcohol, cleared in methyl benzoate, toluene and mounted in paraffin wax. Serial section (7 μ thick) of the selected tissues from livers and kidneys were stained by hematoxylin and eosin for general histology (Drury and Wallington, 1976).

Immunohistochemical investigation

Sections of selected liver and kidney at E14 and E17 of the second main experiment were mounted on Superfrost/Plus glass slides. The slides were deparaffinized in xylene, rehydrated in serial alcohol and retrieved for re-antigenicity using 10 mM citrate buffer at pH6 at 100 °C in oven for an hour (Buchlowalow and Bocker, 2010). Sections were incubated with specific primary antibody against anti-HSP70 (rabbit polyclonal, spring, Bioscience, USA) and BAK (rabbit polyclonal antibody, Santa Cruz, Biotechnology, INC., Germany), for three hours at room temperature. Sections were then washed using phosphate buffer and incubated with secondary antibody (biotinylated goat anti-polyvalent HRP DAB detection system, Spring Bioscience, USA). Then sections were washed with phosphate buffer and then visualized by mix chromogen solution with DAB. Stained sections were mounted with DPX mounting media and investigated under light microscope (Axio Scope, Zeiss, Germany).

Transmission electron microscope study

Liver and kidney samples at E17 from the different subgroups of the second main experiment were fixated in 2.5% glutaraldehyde (Mercer and Birbeck, 1972), then washed twice in distilled water, then post-fixed in 1% osmium tetroxide, then washed twice in distilled water and dehydrated in ascending series of ethanol. At the stage of clearing, samples were passed in propylene oxide. In the infiltration process samples were transferred to a mixture of propylene oxide with epon resin 1:1. The samples were then embedded in pure epon resin in blocks at 60 °C overnight. Ultrathin sections were cut using ultratome (Reichert, Supernova, Germany) and were mounted in grids, stained with uranyl acetate and lead citrate (Reynold, 1963). Sections were examined and photographed under a Joel Transmission Electron Microscopy (JEM 1010, Japan).

Results

Toxicological observation

The females of verapamil treated groups either once or twice daily that were treated from zero day of gestation showed