Effects of giant honey bee (Apis dorsata) venom on renal failure in rats (Rattus norvegicus)

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ORIGINAL RESEARCH ARTICLE

Effects of giant honey bee (*Apis dorsata*) venom on renal failure in rats (*Rattus norvegicus*)

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In recent decades, massive attacks with several hundred bee stings causing severe injury and inducing acute renal failure have been reported in many countries including Thailand. However, there have been no studies of the effects of giant honey bee *Apis dorsata* stings on animal tissue. This study thus aimed to investigate the effects of *A. dorsata* venom on renal failure in male rats (*Rattus norvegicus*) using three dosages of venom prepared by dissolving the venom in .85% NaCl (normal saline): 30.0 μg/g body weight (high dose, HD); 20.0 μg/g body weight (low dose, LD); and .0 μg/g body weight for the control (CO). The rats were injected with 200 μl of each dose. After 24 h, renal tissues were collected and processed by paraffin technique, stained with periodic acid-Schiff's reagent and Masson's trichrome stain for histological structure analyses. The findings showed that Bowman's space of HD rats was significantly narrower than those of LD and CO ($F_2 = 3.94$, $p \le .0201$). Moreover, the percentage of destructural glomeruli of HD was significantly higher than those of LD and CO, these were 44.14 ± 2.41%, 29.65 ± 1.20%, and 10.76 ± 1.74%, respectively ($F_2 = 676.02$, $p \le .0001$). In addition, the HD rats also showed the highest injury of brush borders of proximal convoluted tubules ($F_{2,4} = 848.09$, $p \le .0001$). The impaired tubular cell nuclei were observed in the dosed groups. These results indicate that experimental doses of bee venom can cause signs of renal failure in the rat model.

Efectos del veneno de la abeja gigante (*Apis dorsata*) sobre la insuficiencia renal en ratas (*Rattus norvegicus*)

En las últimas décadas, se han documentado en muchos países, incluyendo Tailandia, ataques masivos con cientos de picaduras de abeja responsables de lesiones graves y de inducir insuficiencia renal aguda. Sin embargo, no se han realizado estudios de los efectos de las picaduras de la abeja gigante *Apis dorsata* en los tejidos animales. Este estudio tiene como objetivo investigar los efectos del veneno de *A. dorsata* sobre la insuficiencia renal en ratas macho (*Rattus norvegicus*) utilizando tres dosis de veneno preparadas disolviendo el veneno en NaCl (solución salina normal) al 0,85%: 30,0 μg/g de peso corporal (dosis alta, HD); 20,0 μg/g de peso corporal (dosis baja, LD); y 0,0 μg/g de peso corporal para el control (CO). Se inyectaron 200 μl de cada dosis en ratas. Después de 24 horas, los tejidos renales se recogieron y se procesaron por la técnica de la parafina, se tiñeron con ácido peryódico-reactivo de Schiff (PAS) y con la tinción tricrómica de Masson para los análisis de la estructura histológica. Los resultados mostraron que el espacio de Bowman de las ratas del grupo HD fue significativamente más estrecho que el de las ratas de los grupos LD y CO (F_2=3,94, $p \le$0,0201). Por otra parte, el porcentaje de glomérulos con daños en su estructura en el grupo HD fue significativamente mayor que en los grupos LD y CO, estos fueron 44,14±2,41%, 29,65±1,20% y 10,76±1,74%, respectivamente (F_2=676,02, $p \le$0,0001). Además las ratas del grupo HD también mostraron mayores lesiones en los bordes en cepillo de los túbulos contorneados proximales ($F_{2,4}$=848,09, $p \le$0,0001). En los grupos que recibieron la dosis se observaron deteriorados los núcleos de las células tubulares. Estos resultados indican que las dosis experimentales de veneno de abeja pueden causar signos de insuficiencia renal en el modelo de rata.

Keywords: *Apis dorsata*; bee venom; renal failure; rat; *Rattus norvegicus*

Introduction

In Thailand, combs of the giant honey bee *Apis dorsata* Fabricius 1793 are widespread throughout rural areas, but there are sparse data available in the literature on associated dangers of this bee. Bee stings can cause many harmful reactions in humans, including skin necrosis, shock hypertension, bleeding, hemolysis, thrombocytopenia, pancreatitis, (adult) respiratory distress syndrome, rhabdomyolysis, and acute renal failure (ARF) (Mejia, Arbelaez, Henao, Sus, & Arango, 1986). Further, there have been several reports of ARF following honey bee stings (Humvel, Bollandaad, & Hulin, 1998).

There are, however, no epidemiological studies that report multiple bee stings of any bee species associated with induced ARF or rhabdomyolysis in the Thai population. There are several studies that have reported on the effects of honey bee venom on humans (Grisotto et al., 2006). The incidence of anaphylaxis caused by insect stings has been estimated to be from .3 to 3% in the general population (Lee et al., 2007; Palma, 2006;

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Schmidt, 1995). Allergic manifestations to honey bee and wasp stings are well recognized, but more serious complications like intravascular hemolysis, rhabdomyolysis, thrombocytopenia, ARF, liver impairment, and myocardial infarction are less common (Ownby, Powell, Jiang, & Fletcher, 1997; Rojas, Rodriguez-Acosta, Finol, Cespedes, & Hernandez, 2002). ARF would occur due to toxic-ischemic-type mechanism as hypovolemia, myoglobinuria, hemoglobinuria, renal ischemia, or direct venom toxicity (Deshpande, Tarooq, Bairy, & Prabhu, 2013; Ownby et al., 1997). It has been observed that multiple bee stings are capable of causing death, in an adult man, which is probably due to a direct toxic effect of the venom (Habermann, 1972; Rojas et al., 2002).

The aim of this study was to examine the effect *A. dorsata* venom at dosages of 20.0 and 30.0 μg/g body weight on renal failure of male rats via analyses of the histological structure of rat kidney.

Materials and methods

Animals

Adult male Wistar rats (*Rattus norvegicus* Berkenhout, 1769) weighing on average 103.1 ± 2.6 g were housed at 25 °C with a photoperiod of 12:12 (L: D). Standard diet and distilled water were provided.

Bee venom

Venom was extracted from 70 guard bees of *A. dorsata* (average weight of venom glad was .45 ± .35 mg) collected from Karasin province, Thailand, and the crude extract was dissolved in .85% NaCl (w/v) for two doses and a control: 30.0 μg/g body weight (high dose, HD; *n* = 3), 20.0 μg/g body weight (low dose, LD; *n* = 3), and .0 μg/g body weight for the control (control, CO; *n* = 3).

Tissue preparation and renal failure studies

Male rats were injected with 200 μl of each dose via subcutaneous tissues. After 24 h, the renal tissues were fixed with Bouin's fluid solution, dehydrated with serial dilution of ethanol 70–100%, and embedded in melt paraplast at 56 °C. Tissues were sectioned for 6 μm thick sections using a rotary microtome (Leica; Germany), stained with periodic acid-Schiff's reagent (PAS) and Masson's trichrome stain for histological structure analyses, and evaluated by light microscopy, every rat the microscopic sections through kidneys are similar with respect to the position and area within the kidney.

Destructural glomeruli

The width and the length of glomeruli and Bowman's space were measured using a micrometer; *n* = 30 per rat each dose.

Brush border injuries

The injury of brush border at the free end of proximal convoluted tubules was estimated for five levels of injuries; 0% was the normal structure without any tissue damage at the free end of proximal convoluted tubules; 1–25% was the tissue damaged with less than 1/4 of the area of free end of proximal convoluted tubules; and 26–50%, 51–75%, and more than 75% of damaged tissues were found, *n* = 30 per rat each dose.

Statistical analyses

Data were expressed as mean ± SE (standard error of mean). The significance of difference means of experimental groups was analyzed using one-way and two-ways ANOVA and Duncan's multiple range test.

Results

Renal histology

A. dorsata venom at the dosage of 30.0 μg/g body weight was associated with glomerulus dispersion, the narrowest of Bowman's space, and the highest injury of brush

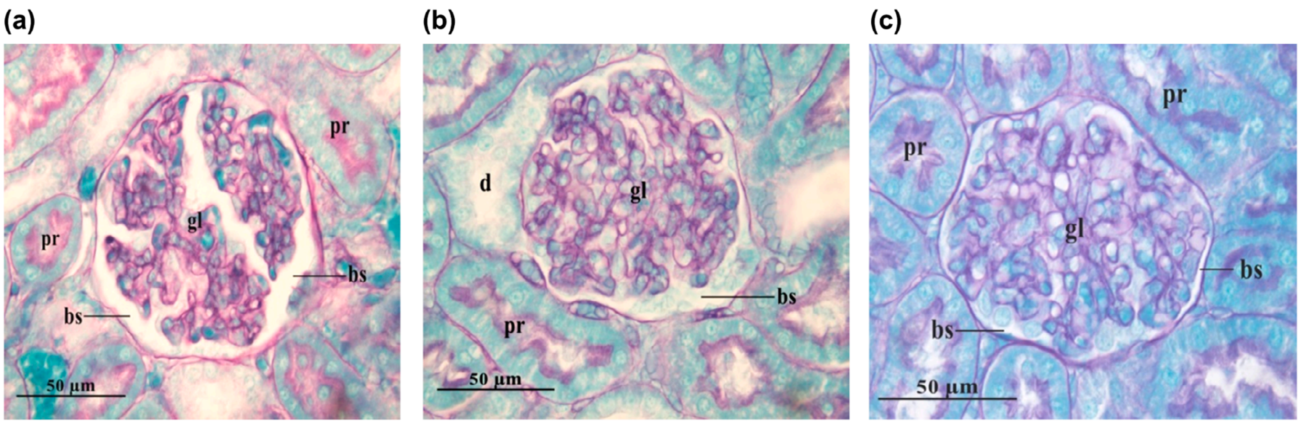

Figure 1. Light micrographs of nephrons of HD (a), LD (b), and CO (c). 1000x, PAS; bs, Bowman's space; d, distal convoluted tubule; gl, Glomerulus; pr, proximal convoluted tubule.

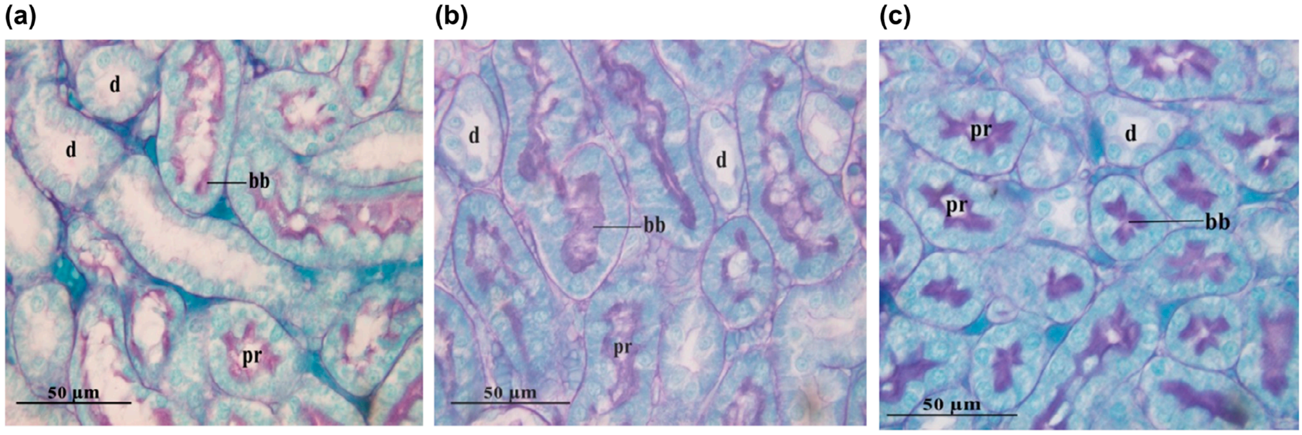

Figure 2. Light micrographs of proximal convoluted tubules of HD (a), LD (b), and CO (c) showing different injury levels of brush border. 1000x, PAS; b, brush border; d, distal convoluted tubule; pr, proximal convoluted tubule.

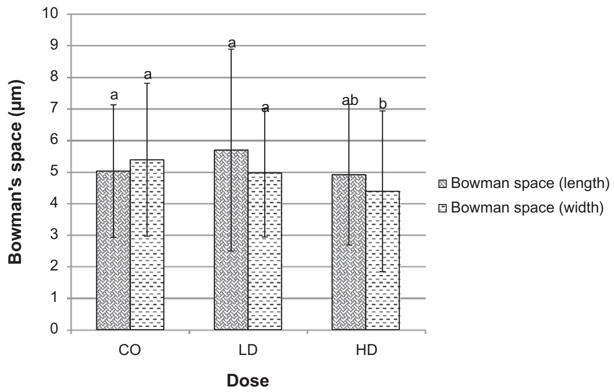

Figure 3. The width and the length (mean ± SE) of Bowman's space of rat dosed with 30.0 µg/g body weight (HD); 20.0 µg/g body weight (LD), and normal saline (CO); vertical bars with different letters represent significant difference, $F_{2,1}$ = 3.94, .20, $p \leq .0201$).

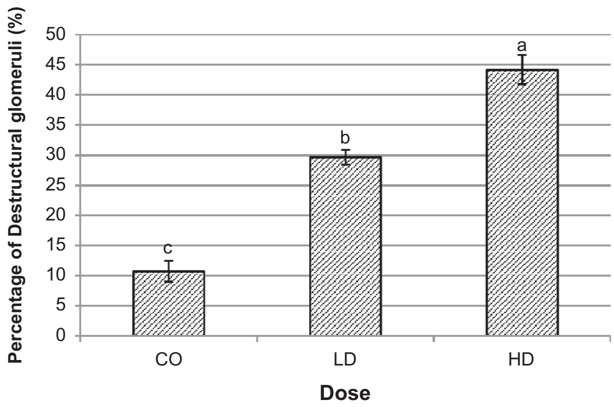

Figure 4. The percentage (mean ± SE) of destructural glomeruli of rats dosed with 30.0 µg/g body weight (HD); 20.0 µg/g body weight (LD), and normal saline (CO); vertical bars with different letters represent significant difference, F_2 = 676.02, $p \leq .0001$).

borders of proximal convoluted tubules (Figure 1(a) and 2(a)). At the dosage of 20.0 µg/g body weight, there was dispersion of glomerulus structure and injury of brush border (Figure 1(b) and 2(b)). However, no histological changes were found in the control groups (Figure 1(c) and 2(c)).

Destructural glomeruli and brush border injuries

The Bowman's space of HD rats was significantly narrower than that of LD and CO rats (F_2 = 3.94, .20, $p \leq .0201$) (Figure 3). Moreover, the percentage of destructural glomeruli of HD was significantly higher than those of LD and CO, which were 44.14 ± 2.41%, 29.65 ± 1.20% and 10.76 ± 1.74%, respectively (F_2 = 676.02, $p \leq .0001$) (Figure 4). In addition, the HD rats also showed the highest injury of brush borders of proximal convoluted tubules ($F_{2,4}$ = 8.51, 848.09, $p \leq .0001$) (Figure 5). The impaired tubular cell nuclei were found in

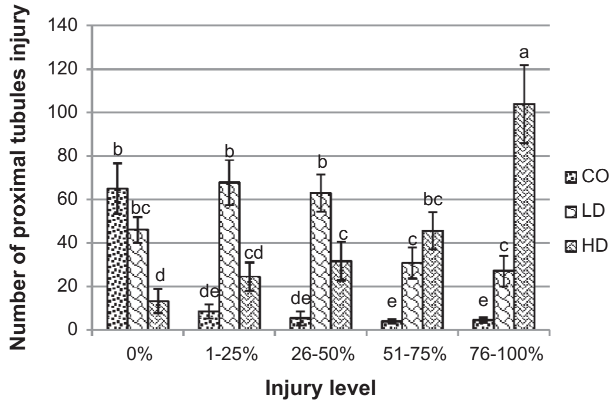

Figure 5. The number (mean ± SE) of proximal tubules suffering categories of injury levels (%) of brush border at the free end of proximal convoluted tubules of rat dosed with 30.0 µg/g body weight (HD); 20.0 µg/g body weight (LD), and normal saline (CO); vertical bars with different letters represent significant difference, $F_{2,4}$ = 8.52, 848.09, $p \leq .0001$).

the dosed groups. These results indicate that the experimental dose of bee venom can cause signs of renal failure.

Discussion

The results of this study provide new aspects of bee venom of *A. dorsata* inducing renal injury in male rats. A sac of venom of this species contains around .45 mg of venom, while that of Africanized honey bee contains .10 mg (Schumacher, Schmidt, Egen, & Lowery, 1990). Therefore, an incident with 6–8 stings would deliver approximately 30 µg/g of venom to an individual weighing 100 g, or 30 mg/kg of venom in human individual of 100 kg. Thus, for the average human body weight that is about 60 kg, this dosage would require about 360–400 stings delivered into human body. However, giant honey bee stings received by Thai bee hunters could result in either mild clinical symptoms that accompany pain, or could cause death by anaphylactic reaction in some people, because its toxin can cause organ dysfunction and multiple organ failures. In human studies, the HD showed that the injury of brush border and glomeruli corresponds to observations in patients with bee venom-induced ARF, i.e., an important and early GFR and diuresis decreases. In fact, there are clinical reports of oliguria (urine volume) and serum creatinine increase within 2 h after the sting (Franca et al., 1994). Early histology disclosed retraction of the glomerular tuft and mild acute tubular injury. Glomerular retraction is usually associated with glomerular ischemia, which is consistent with the striking RBF decrease observed after venom infusion. After 24 h, the lesion evolved to a frank tubular injury, which is the lesion found in renal biopsies or autopsies of patients with bee venom-induced ARF (Prado, Solano-Trejos, & Lomonte, 2010).

The main component of this venom is melittin which is a toxic surface polypeptide that might be related to RBF impairment in many ways. It can damage the vascular endothelium, cause vasoconstriction, smooth muscle cells contraction (Franca et al., 1994), and increased renal renin secretion. Further, phospholipase A_2 accounts have been related to diverse situations of intra-renal vasoconstriction (Grisotto et al., 2006). These results indicate that the toxin of *A. dorsata* is capable of producing renal dysfunction as well as producing allergic reactions, which vary from a mild reaction to fatal reaction reported from time to time in many countries (Deshmukh & Borse, 1996).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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