

REVIEW A RTICLE

VENOMOUS SALIVA OF NON-HAEMATOPHAGOUS REDUVIID BUGS (HETEROPTERA: REDUVIIDAE): A REVIEW

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ABSTRACT

While reduviids are a modestly well characterized group of insects, especially the blood sucking triatominae due to the medical implications of the Chagas disease, which is mainly transmitted by the infected bugs whose excrement contains *Trypanosoma cruzi* that enters the body through bruises or cuts in the skin of humans, their non-haematophagus counterparts are a forgotten lot and have not been thoroughly investigated. The venom in the saliva of the non-haematophagus reduviids has come into the spotlight in the last couple of decades due to the voracious predatory lifestyle that enable them to be used as biological control agents in subduing pests. But the biochemistry of reduviid venom, its action and subsequent effect on the prey, toxicity, enzymes, peptides present in the venom and their significance, the role of extra oral digestion facilitated by the venom for its predatory lifestyle have not been given much consideration. This review aims to summarize the existing body of literature regarding the venomous saliva of non-haematophagous reduviid bugs for the first time.

Key words : reduviid bugs, enzymes, peptides, toxin, venomous saliva.

INTRODUCTION

Reduviid bugs constitute one of the largest and most successful groups of predacious insects, cosmopolitan in occurrence with approximately 6800 species (Hwang and Weirauch, 2012; Maldonado, 1990) and ecological specializations with diverse, highly evolved prey capture strategies (Soley *et al.*, 2011; Jacobson, 1911; Zhang and Weirauch, 2011; Wignall and Taylor, 2011; Ferero *et al.*, 2011) and the description and redescription of many species is being constantly incorporated at the species, generic and subfamily levels to the already existing exhaustive list (Ambrose, 1999; 2004). Most assassin bugs exhibit generalist predation wherein they prey on other arthropods, while others show specialist predation by preferring to feed on a certain group of prey organisms such as termites, ants and diplopods (Jacobson, 1911; Louis, 1974; Cobben, 1978; Weirauch and Cassis, 2006, McMahan, 1983a; 1983b; Miller, 1953). The salivary secretions of these bugs play a pivotal role in feeding as they can only ingest liquid food. It is at this juncture that the saliva begins to assay the function of a venom thus enabling reduviids to extensively exploit their predatory behavior and evolve innovative methods of predation (Edwards, 1961). Hence, the saliva of reduviid bugs is often called venomous saliva.

Venoms are natural toxins with a cocktail of complex compounds that can serve as a great

source of novel bioactive substances with unrealized potential application in the field of drug discovery, medicine and agriculture. Besides the extensive work on the blood sucking triatominae [Anderson *et al.*, 2003; Amino *et al.*, 2002; Noeske-Jungblut *et al.*, 1994; Goodchild, 1955; Wigglesworth, 1943; Schofield, 2000; Teo and Cheah, 1973; Schofield, 1994; Sandoval *et al.*, 2000; Ryckman, 1951; Patterson, 1999; Miles, 1981; Lent and Wygodzinsky, 1979; Guerenstein and Guerin, 2001], the venomous saliva of predatory reduviid bugs has not been given due consideration.

Studies on the biochemistry and composition of reduviid bug venoms are meager and have been confined to the following species: Acanthaspis pedestris Stal [Morrison, 1989], Peirates turpis Walker, Agriosphodrus dohrni Stal and Isyndus obscurus Dallas [Gerardo et al., 2001], Peirates affinis Serville [Edwards, 1960], Platymeris rhadamanthus Gerstaecker [Edwards, 1961], Holotrichius innesi Horrvath [Zerarchia et al., 1973], Zelus renardii Kolenati [Cohen, 1993], Haematorrhophus nigroviolaceus Reuter [Haridass amd Ananthakrishnan, 1981]. Catamirus brevipennis Serville [Sahayaraj et al., 2007] and *Rhynocoris marginatus* Fabricius [Sahayaraj et al., 2013]. It is the purpose of this paper to review and summarize the existing body of literature on the venomous saliva of nonhaematophagous reduviid bugs.

REVIEW

Effect of venomous saliva on the prey:

Reduviid bugs have an elongated head with a distinct narrow neck, long legs and a prominent segmented rostrum for feeding (Hilty, 2013; Ambrose, 1999). After capturing the prey, these bugs use the long rostrum to inject toxic saliva that liquefies the insides of the prev which are later sucked out (Jacobson, 1911; Edwards, 1961; Sahayaraj, Haridass 1994; and Ananthakrishnan, 1980; Cohen, 1990). The bite of the reduviid bug causes intense localized pain and swelling and leaves a long-standing discolored or blackened pit at the point of insertion of the rostral stylets. The dried saliva powder of these bugs exhibits an irritant activity

with respect to eye and nose membranes, and induces oedema, vasodilation, increased mucous secretion and respiratory disturbances similar to those caused by snake venom and aids in the initiation of various allergic reactions (Stanic, 1956). The saliva which enters the body of the prey after the painful bite contains enzymes that digest the tissues the predators swallow, a process known as extra-oral digestion. This phenomenon is found to be ecologically important as it allows relatively small predators to consume large prey that cannot be swallowed or ingested as a whole. This characteristic of the saliva renders the bug highly effective at killing prey much larger than itself (Haridass and Ananthakrishnan, 1980; Cohen, 1990).

The venomous saliva paralyzes the prey within a short duration of time, after which the bug uses its fore legs to hold the prey and suck its bodily juices. The first instar larva of Rhinocoris carmelita Stal is able to paralyze a final instar larva of Ephestia kuehniella, over 400 times its own weight within a limited duration of 10 seconds and **Platymeris** rhadamanthus Gerstaecker immobilizes the cockroach Periplaneta americana Linnaeus within 3-5 seconds and stalls its struggling (Edwards, 1961). In the case of successful insertion of rostral stylets and injection of salivary toxins, larger prey such as millipedes, caterpillars and beetles become completely paralyzed within 20-30 seconds (Haridass, 1985). In view of the rapid neurotoxin induced death of the prey as seen in the above mentioned examples, it is interesting to note the observations made by Sahayaraj and Vinothkanna, 2011, wherein they state that reduviid bug venoms cause long term, non lethal paralytic effects on their prey.

The salivary fluids are usually injected into the prey, with the favored site of injection being the legs, neck and antennae (Haridass and Ananthakrishnan, 1980; Cohen, 1990). The movement of the prey's stylet causes the mixing of the prey tissue and the predator's saliva after which the mixture is sucked out. This behavior was termed as lacerate-and-flush feeding by Miles, 1972. This technique enables the predators to intake large amounts of digestible prey material without ingesting non-digestible matter. Very often, only a portion of the nutritional parts of the prey are ingested, a process referred to as partial consumption of prey, commonly observed in various heteropterans including reduviid bugs (Lucas, 1985). Although the prey are only partially consumed, these predators are highly efficient at prey extraction, ingesting more than 80% of the prey nutrients and absorbing more than 90% of what they ingest (Cohen, 1989). Thus, due to external digestion facilitated by the toxic saliva, removal of 40 to 60% of the prey's live weight, at the rate of 1.5 to 2 mg per minute is effected (Edwards, 1961) with one complete feeding session with an immobilized prey lasting for about 90-120 minutes (Haridass and Ananthakrishnan, 1980; Cohen, 1990). Injecting venom into the prey, which is a process of prey preparation, expands the predatory scope or the effective size range of prey that can be exploited (Hespenheide, 1973). This renders reduviid bugs as ideal candidates for biological control agents in places where a wide variety of insect pests need to be subdued. Their distinct character of indiscriminate killing wherein they kill more prey than they need to satiate themselves also reinstates their yet to be tapped, immense potential as effective biological control agents.

Collection of venomous saliva from Reduviid bugs:

Edwards in 1961 successfully used the method of intimidation with an unknown object to extract venom. He used a seeker to molest the insect by tapping its thorax through the break in the petri dish. This induced the bug to spit saliva from its rostrum onto the glass above. This saliva when dry is scraped from the surface using a steel blade. Venom was also obtained by holding the abdomen of the predator between the thumb and the index finger and by gently pressing the abdomen. This action stimulates the insect to eject venom which is then collected in a capillary tube that was inserted in the rostral tip. The insect on subjection to an electric stimulus of about 180-230 volts also ejects venom (Sahayaraj et al., 2006). The whole body extracts of the insect and dissection of venom or salivary gland also yields venom. But these methods are

rarely used as they give a very crude preparation (Ambrose and Maran, 1999). The salivary secretions can also be gathered by placing the labium of the predator in a capillary tube and injecting the insect with 2 μ l of a 0.5% solution of pilocarpine which causes excessive and spontaneous salivation (Cohen, 1990).

Role of enzymes in EOD:

Digestion, in non-haematophagous reduviids is a highly efficient process that is categorized into two phases: pre-oral digestion that takes place outside the body and actual digestion in the gut of the insects. The process begins with pre-oral digestion of the prey's internal structures whose venom enabled liquefaction permits ingestion followed by further digestive processing of prey within the predators gut. In reduviids and all true enzymes which are produced bugs. in specialized glands are forced into the prey and ingested into the predator's gut, where they remain until digestion is complete. This pre-oral digestion is a form of food preparation (Kaspari, 1990) that helps in the intake and eventual consumption of prey which are too large to digest. Nutrient intake in the form of EOD is a cyclical and incremental process, i.e. after the prey has been brought under the control of the predator, a series of injections of digestive fluids in successive bouts interspersed by regular intervals is pumped into the prey followed by a mechanical pause after which ingestion of the disgorged fluids and portions of the liquefied prey take place (Cohen, 1993). This method of cycling increases the efficiency of the process of digestion by maximizing the concentration of hydrolytic enzymes in proportion to the volume of prey to be liquefied (Cohen, 1984, 1989; Baptist, 1941).

EOD is completely facilitated by digestive enzymes but the origin of these enzymes has been a matter of debate for many years. Even though studies have reported the presence of proteinase activity in the salivary secretions of many insects including reduviids (Edwards, 1961; Rastogi, 1962; Rees and Offord, 1969) there is a general view that salivary secretions are of have no significance outside of the predators' digestive system. Baptist in 1941, recorded the presence of proteinase in the salivary secretions of predaceous heteropterans, but claimed that these enzymes worked too slowly to be of any importance. Many including Law et al., 1977 have expressed doubt and concern in relation to the salivary glands being considered as the source of proteolytic enzymes injected by carnivorous insects into their prey. These doubts may have arisen from the fact that in certain predatory arthropods that use pre-oral digestion, like the carabid beetles, the source of enzymes is the gut and not the salivary glands (Cheeseman and Gillott, 1987). The work done by Cohen in 1990, wherein radio labelled inulin was used to trace the origin of the enzymes, demonstrated that the gut was not the source of disgorged digestive enzymes. Moreover, a large amount of identified routine digestive enzymes that were present in the guts of the predators never reached the prey. Preliminary work on the digestive enzyme elastase, an alkaline proteinase that complements the actions of trypsin and chymotrypsin, also reveals that reduviid predators have this enzyme present in their salivary system (Cohen, 1998). In addition, Haridass and Ananthakrishnan in 1981, dissected the anterior and posterior lobes of the salivary glands from certain specific reduviid bugs such as Haematorrhophus nigroviolaceus Reuter, Fabricius, Ectrychotes Guionius nigripennis pilicornis Fabricius, Pirates affinis Serville, Ectomocoris vishnu Distant, *Catamiarus* brevipennis Serville, Triatoma rubrofasciata De Geer, Linshcosteus costalis Ghouri, Acanthaspis Distant. Acanthaspis pedestris siva Stal. Acanthaspis quinquespinosa Fabricius, Lizarda annulosa Stal, Petalochirus indicus Reuter, Rhaphidosoma atkinsoni Bergroth, Sycanus collaris Fabricius, Sphedenolestes bowringi and tested the efficacy of their Distant homogenates on prospective prey. Immediate and rapid paralysis of the prey proves the zootoxic effects of the salivary secretions. These experiments clearly show that the gut is not the source of digestive enzymes injected into the prey which facilitate external digestion. In fact, not all secretions of the salivary gland are toxic.

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salivary gland complex function as water recapturing agents (Miles, 1972; Goodchild, 1966). The enzyme profile of some reduviid bug salivary secretions is given in table 1.

The biochemical differences between venoms and digestive secretions are difficult to resolve, especially since many venoms are seen to originate from digestive system structures (Scmidt, 1982). The most abundant enzymes that were present in the salivary secretion include proteinases, amylase which hydrolyzes starch to form maltose and is useful in the digestion of glycogen, invertase which hydrolyses sucrose to form fructose and glucose, lipase which splits fats into fatty acids and glycerol, pepsin and trypsin which break down complex proteins into peptones (Swingle, 1925). Proteinases are the important liquefaction most enzymes for predators (Cohen, 1993; Miles, 1972; Rees and Offord, 1969). Proteinases are classified into endopeptidases and exopeptidases. Endopeptidases attack protein molecules from within, reducing insoluble structures into watersoluble subunits. Trypsin like enzymes attack proteins at their basic amino acid sites, cleaving the proteins at lysine and arginine residues (Law et al., 1977). Liquefaction results from the endopeptidase activity of the saliva. Chymotrypsin like enzymes attack proteins at their aromatic sites (Gilmour, 1961, Cohen, 1993). Phospholipase found in the saliva digests phospholipids in cell membranes, disrupting neurons and muscle cells (Schmidt, 1982). Hyaluronidase is a well known spreading factor for venoms that aids in quick paralysis of the prey (Mommsen, 1978; Foelix, 1982; Schmidt, 1982).

The enzymes are an indispensable resource that must be given approximate time to liquefy prey and must be recovered for further use in the gut if the predator is to exploit their full value. These enzymes cannot be immediately replaced if are lost or emitted unnecessarily. The specificity of these enzymes determines that only certain structures are liquefied and made available for ingestion. As a direct resultant action of the selective nature of these enzymes, the predator ingests only the inner contents of the prey and

The anterior lobe secretes neurotoxic substances,

while the posterior lobes secrete digestive

enzymes. The accessory glands present in the

Peptides in reduviid bug saliva:

(Morrison, 1989; Maran, 2000) peptides

Reduviid bug venomous saliva is known to

contain a complex mixture of proteins

thus, they have the advantage of selecting nutrient rich food unburdened by indigestible and potentially damaging cuticular structures (Hespenheide, 1973).

Table 1. Enzyme profile of reduviid bug saliva

	Platymeris rhadamanthus	Rhynocoris marginatus	Catamiarus brevepennis	Zelus renardii	Sinea confusa
Proteinase	+	+	+	+	+
Hyaluronidase	+	+	+	*	*
Lipase	-	+	+	+	+
Esterase	-	+	+	*	*
Phospholipase	+	+	+	+	+
Adenosine	+	+	+	+	+
triphosphate					
Amylase	+	+	+	+	+
Invertase	+	+	+	*	*
Trypsin	+	+	+	*	*
Pepsin	+	+	+	*	*
Acid phosphatase	+	+	+	*	*

+ Present; - Absent; * Not known

Table 2. Toxicity of reduviid bug salivary gland homogenates

Species	Prey	Action
Platymeris rhadamanthus	Periplaneta americana	Immediate cessation in systole;
		general contracture
Pirates affinis	Omphora pilosa	Immediate stoppage of all body
		movements and total paralysis
Rhynocoris carmelia	Ephestia kuhniella	Immediate cessation in systole;
		general contracture
Reduvius personatus	Periplaneta americana	Immediate cessation in systole;
		general contracture
Naucoris cimicoides	Periplaneta americana	Immediate cessation in systole;
		general contracture
Oncopeltus fasciatus	Periplaneta americana	Slow decrease in amplitude;
		slight increase in rate, cessation
		after some minutes
Pentatoma rufipes	Periplaneta americana	Slow decrease in amplitude;
		slight increase in rate, cessation
		after some minutes
Haematorrhophus nigroviolaceus	Xenobolus carnifex	Quick stoppage of antennal and
		leg movements and total paralysis
Platymeris rhadamanthus	Periplaneta americana	Immediate cessation in systole;
		general contracture
Pirates affinis	Omphora pilosa	Immediate stoppage of all body
		movements and total paralysis
Rhynocoris carmelia	Ephestia kuhniella	Immediate cessation in systole;
		general contracture

(Gerardo et al., 2001) and enzymes (Sahayaraj et al., 2007; 2011; Edwards, 1961). MALDI-TOF screening of the salivary secretion of the reduviid bugs Peirates turpis, Agriosphodrus dohrni Stal and Isyndus obscurus Dallas, followed by HPLC fractionation yielded components having molecular mass from 2 kDa to 30 kDa. Upon further purification, 3 peptides designated as Ptu1, Ado1 and Iob1 with molecular masses of 3615.3, 3781.2 and 3938.6 Da were identified from *Peirates turpis*, Agriosphodrus dohrni Stal and Isyndus obscurus Dallas respectively. Although the three peptides were from different reduviid bugs, their amino acid sequence motif was well conserved with some point mutations and was relatively homologus to the Conotoxins (Gerardo et al., 2001).

Conotoxins are venom ejected by *Conus* species to help hunt their prey. A single injection can cause the fish to be immobilized within 1 or 2 seconds. Total paralysis was effected a few seconds later. It has been observed that the biologically active small peptides in the venom contribute to its potency (Olivera et al., 1991). These paralytic elements could well be those responsible for the action of reduviid venom. Similarly mass spectrometric analysis of *Rhynocoris* marginatus saliva contained components with molecular masses ranging from 3 kDa to 50 kDa. Three peptides namely RmIT-1, RmIT-2 and RmIT-3 with a molecular mass of 3.79, 7.5 and 10.94 kDa were identified (Sahayaraj et al., 2013). Peptides identified from Rhynocoris fuscipes include RfIT1 and RfIT2 with 2358 and 3423 Da molecular mass respectively (Sahayaraj, 2013).

pH of venomous saliva:

The freshly secreted venomous saliva of the reduviid bugs has pH ranging from 6.6 to 6.8 with that of adult females slightly alkaline when isolated from recently prey fed individuals. However, the saliva is found to become gradually neutral after three days of food deprivation and continues to become more acidic with increasing length of the starvation period (Sahayaraj *et al.*, 2013; Edwards, 1961). The pH of the prey was found to be between 7.0 to 7.2

during the initial stages of feeding and continued to increase till 8.5 after which it kept fluctuating till the end of feeding but never touched below 7.5 (Cohen, 1993).

Action of venomous saliva on whole animals, organs and tissues:

The venomous saliva of reduviid bugs have proved to be toxic to a wide range of insects representing seven orders but are immune to their own species. Application of saliva obtained from the adult insects on the heart of the younger instars of the same species show no marked alteration in their rhythmic contractions. 5 to 15 % of the saliva application on the heart dorsum of an arthropod prey brings about immediate cessation of the systolic movements followed by a general contracture. 5 to 10 % of saliva when applied on the abdominal nerve causes an increase in electrical activity for a few seconds which terminates abruptly after which the nerve cord ceases to conduct. With regard to muscles, the saliva caused an immediate strong coiling followed by slow uncoiling with concurrent lysis of tubule cells at higher concentrations. The contractions become irregular, with movements eventually ceasing either in the coiled or extended state.

The lytic activity of the venomous saliva is highly pronounced with breakdown of fat bodies being the first effect after the paralysis of the prey. Major changes in the appearance and mechanical properties of the tissue are apparent and observable within a short time of immersion in insect saliva (Smith and Wigglesworth, 1959; Wigglesworth. 1957). The responses of innervated and non-innervated muscle, intact and isolated nerve to treatment with the venomous saliva indicate that the mechanism of paralysis does not involve a specific site of action. Rather, it has been observed that the saliva attacks and disrupts the cell membranes on which the functioning of the excitable tissue depends. The subsequent lysis that occurs is an extension of the initial membrane breakdown that causes paralysis. In other words, the paralysis caused by the reduviid bug saliva is a special function of external digestion (Edwards, 1961).

Toxicity of reduviid bug venomous saliva:

The salivary system of reduviid bugs is very complex (Southwood, 1955, Haridass, 1978; Baptist, 1941). The anterior lobes of the main glands are concerned with the secretion of neurotoxic substances involved in the paralysis and death of the prey while the posterior lobes secrete the digestive enzymes. The venomous saliva in the dried state retains toxicity for at least three years but declines slowly in potency in aqueous solution. The saliva of Pirates affinis and Haematorrhophus nigroviolaceus exhibit pronounced zootoxic effects. The salivary gland homogenates of P. affinis and H. nigroviolaceus cause total paralysis and complete stoppage of all twitching movements of the carabid beetle in 12-16 seconds. Similar effects were seen with the homogenates of H. nigroviolaceus on the movements of the millipede in 48-52 seconds (Haridass and Ananthakrishnanm 1981). In table 2, the toxicity of some bugs and their resultant effect on the prey are tabulated.

C. brevepennis venom shows antibacterial activity against the pathogens Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Staphylococus aureus, Bacillus sphaericus and Salmonella typhimurium, while R. marginatus venom shows the same activity against only Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella *Streptococcus* pyogenes and typhimurium (Sahayaraj et al., 2006). The saliva of R. fuscipes was found to be venomous to Helicoverpa armigera and Spodoptera litura when the larvae were treated orally or by injection. It has also been noted that the crude venom has more impact than purified peptides and that the toxic nature of venomous saliva is due to its protein content (Sahayaraj and Vinothkanna, 2011). The protein content of the saliva varies with respect to males and females. In *R. marginatus*, the protein content was $1.16 \pm$ 0.03 mg/100 mg body weight for males and 0.92 \pm 0.02 mg/100 mg body weight for females (Sahayaraj et al., 2013). This implies the existence of difference in salivary production and action with respect to male and female reduviid bugs. Records of the female bug paralyzing the prey more rapidly than the male reinstates the fact that the toxicity of the saliva is also seen to vary in adult male and female insects (Sahayaraj *et al.*, 2007).

CONCLUSION

Reduviid bug salivary venom has long been hypothesized to facilitate external digestion following the immobilization of the prey. Research developments have clearly established that the enzymes that bring about external digestion are being produced and secreted from the lobes of the salivary gland rather than the gut. Evidence for the digestive role of the venoms is provided by the extensive tissue damage that occurs in the prey after a bite. Thus it can be stated that either salivary venoms were evolutionarily selected to complement the digestive process or normal saliva could have evolved into its venomous counterpart as an adaptation towards a more successful predatory technique or prey capture strategy. Either way, the venomous saliva in non-haematophagous reduviid bugs is proving to be highly effective in using these bugs as biocontrol agents (Anand et al., 2010; Imamura et al., 2008; George and Ambrose, 2001; Claver et al., 2002; 2003; Grundy, 2007; Vennison and Ambrose, 1992; Wignall and Taylor, 2011; Edwards, 1962; Nagarajan and Ambrose, 2013; Ambrose and Kumaraswami, 1990; Lakkundi, 1989; Evangelin et al., 2012; Claver et al., 2004; Rocha and Redaelli, 2004). While extra oral digestion employed by the reduviid bugs increases the maximum size of the prey that a given predator can handle, it does not compromise the predator's ability to handle prey at the smaller end of its prey range (Nentwig and Wissel, 1986). Due to this typical characteristic, reduviids may not be useful as predators on specific pests, but are valuable predators in situations where a variety of insect pests occur (Schaefer, 1988). They can be efficiently mass reared and disseminated in the pest infested fields with ease. This process can also be suitably customized as per individual requirements and incorporated into integrated pest management strategies. With their large and diverse size range in addition with their fascinating specialized habits aided by venomous saliva. reduviid bugs have considerable but unrealized potential as biological control agents.

Despite the apparent lack of literature on the venomous saliva of non-haematophagus reduviid bugs, we should be extremely cautious with claims regarding their primary biological utility and applicative purposes. More work along these lines can open up new avenues aimed at understanding the toxinology and evolutionary aspects of predatory venoms and can effectively aid in developing novel agrochemicals and pharmaceuticals.

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