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# Pharmacological properties of the repellent secretion of *Zonocerus variegatus* (Orthoptera: Prygomorphidae)

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**Abstract:** The odours of the whole body and the secretion of *Zonocerus variegatus* were easily recognised and perceived by human volunteers. However, the secretion odour is not related to the odour of the food plant consumed by the grasshopper. The repellency of *Z. variegatus* becomes more pronounced in the 6th. and adult instars whose gland lumens contain an appreciable volume of secretion. The secretion odour is so strong that even dilution does not affect its repulsiveness to humans. The secretion had pharmacological properties: it induced contraction in rat (*Rattus rattus*) stomach smooth muscle preparations and guinea pig *(Cavia porcellus)* ileum, and induced oedema formation in the rat hind paw. The secretion was not lethal to the animals used in this study, effects were temporary and recovery occurs after a short time.

**Key words:** *Z.variegatus*, odour, secretions, vertebrate, invertebrate, actions.

## INTRODUCTION

When molested, both sexes and all instars of the variegated grasshopper, *Zonocerus variegatus* (L.) expel an odorous, milky secretions from a gland opening on the dorsal intersegmental membrane between the first and second abdominal tergites (Idowu 1995). The secretion contains proteins, amino acids, glucose, alkaloids and ions (Idowu & Modder 1998). The penetrating and unpleasant odour of the secretion caused the grasshopper to be avoided by vertebrate and invertebrate predators (Idowu 1997).

Injections of secretions of the grasshopper, Poecilocerus bufonis (Fishelson 1960) and *P. hieroglyphics* (Abushama 1970) into mice resulted in their death. The secretion of *P. bufonis* contains histamine which causes the contraction of the smooth muscle of the guinea pig ileum (von Euw et al 1967). The effects of the secretion of *Z variegatus* on organisms under laboratory conditions has not been reported, and there is no data on the perception of the secretion's odour by human beings. All reports on the repellent gland studied merely state that the odour is repulsive or unpleasant to human beings.

## MATERIALS AND METHODS

### Perception of Odour by human beings

For this experiment, human volunteers were

used. Their perceptions of the odour was recorded as one of the follows:

i) Very Strong: The volunteer could perceive the odour immediately the tube containing the test sample was presented.
ii) Strong: when it takes about 5 - 15 seconds for the odour to be recognised.
iii) Weak: when it takes about 20 - 25 seconds for the odour to be perceived.
iv) No effect: The volunteer did not perceive the odour.

The intensity of the odour was alloted 3, 2, 1 and 0 scores respectively. The sets of experiments performed are:

*Insect Body Odour:-* 10 insects of different developmental stages were placed separately in different stoppered conical flask covered with foil paper. The flasks were numbered 1-7 to correspond to the number of *Zonocerus* instars. The flasks were thoroughly shaken before presentation for odour recognition by the human volunteers. The order of presentation of flasks was change from one volunteer to the other. A time lag of 30 minutes was allowed between presentation of samples.

*Secretion Odour:-* Another set of 68 volunteers chosen randomly was presented with sample bottle containing the pure repellent secretion for odour perception. Distilled water was used as the control. Effect of dilution on the recognition of the secretion odour was also carried out. Undiluted and serial dilutions (0.8, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1) of the secretion were presented to another set of volunteers. The volunteers were also asked to compare the odour emanating from the bottle containing the undiluted secretion with that of the body odour of living adult *Z. variegatus*. A time interval of 30 minutes was allowed between presentation of samples. The human volunteers used were between the age of 20-30 years.

***Effects of the Secretion upon animals*:** This effect was tested on different animals by external application and by injection. Different dosages were injected subcutaneously into mice and rats. External application was done by dropping the secretion on the body of ants, termites, mouth parts of the praying mantids and the eyes and skin of mice and rats. The response of the animals was recorded and compared with control animals subjected to the same treatments with distilled water.

**Effects on the hind paw of rats (*Rattus rattus*):** Adult rats (sprague dawley) weighing between 150g and 200g were selected divided into 4 groups based on the treatment below. They were fasted for 12-16 hours before the treatment. The circumference of the hind paw was measured before and after the experiment. Each paw was measured three times and the mean size was obtained.

The hind paw of the rats in the different groups were infected separately with the following:

a) 0.1mm3 of a 1% (V/V) suspension of carrageenin in 0.9% saline solution (a suspension that is known to induce inflammation of the rat paw.)
b) 0.1mm3 of the repellent secretion.
c) 0.1mm3 of the mixture of carragaenin and the secretion.
d) 0.1mm3 of distilled water.

The effect of the injected fluids on the hind paw of the rats was observed by measuring its circumference limb three hours, 6 , 12 and 24 hours after injections.

***Stimulatory action on smooth muscle*:** Strips of smooth muscles of guinea pig ileum (*Cavia porcellus*), rat stomach and rat colon were used. The rats and guinea pig were killed by a blow on the head dissected for the parts were placed in dishes containing Tyrode's solution. The mesentric membranes were trimmed off along the intestine and stomach parts to free the muscles. Tissue threads were attached at each end by inserting a needle through the muscle wall. One end of the thread was tied to a fixed pin in the moist chamber and the other to a lever which has a fine writing point. The effect of the secretion on the smooth muscles strips was recorded using a Kymograph and a

smoked drum, with acetylcholine as standard. The experiment was performed at 37°C in Tyrode's solution through which air was passed. Between successive doses, the tyrode solution was washed off and the muscle allowed to relax for 4-5 minutes.

## RESULTS

Perception of the odour of the body and the secretion of *Z. variegatus* by human volunteers.

There was instant recognition of the odour of the repellent secretion by the human volunteers upon presentation as strong and repulsive. The volunteers were able to perceive the odour of the secretion even when diluted. The perception of the odour showed that the undiluted form was highest, and lowest in the 0.1 level of dilution.

The response of the volunteers showed that *Z. variegatus* produces an odour which is low in the 1st, 2nd, 3rd and 4th instars, intermediate in the 5th and high in the 6th and the adult instars. Result obtained showed that the perception of the body odour of the 6th and the adult by human volunteers was not significantly different (t-test, P>0.005). Observation during the study also showed that people in Ikare, Owo and Oka all in Ondo state of Nigeria where the insect studied is used as a food item (Table 1). They recognised the fact that adult *Z. variegatus* has an odour which according to their description is bad, pungent and repulsive. However, cooked insect donot produces such odour (Table1).The volunteer recorded the perception of the odour of the whole grasshopper's and the repellent

TABLE I

*Perception of the odour of* Z. variegatus *before and after they are cooked.*
N = 70 (100%)

*Percentage of respondents stating how they perceive the odour of* Z. variegatus

| | Before Cooking | | | After Cooking | | |
|---|---|---|---|---|---|---|
| S/N | Smell | No Smell | Do not Smell | Smell | No Smell | Do not Smell |
| Ikare | 72.2 | 19.5 | 8.3 | 0 | 91.7 | 8.3 |
| Oka | 88 | 8 | 4 | 0 | 96 | 4 |
| Owo | 63.6 | 9 | 27.3 | 0 | 72.7 | 27.3 |

secretion as similars and they were not significant different (t-test, P>0.005).

**Effects of the Secretion mice, mantids, ants and termites:** Drops of the secretion applied to the skin of mice and rats produced no noticeable effects, however, the drops into the eyes of the mice and the rats closed their eyes for about 5-10 seconds. When the eyes were opened thereafter no effect was noticed again and they did not close their eyes again or wink.

The ants and termites moved immediately away from the site of the secretion. Thereafter, they wriggled their body for the remaining secretion on their body to fall off. The ants did not allow their antennae to touch the secretion. The movement of the ants and the termites after the secretion was dropped onto their body was low compared to the control animals treated with distilled water.

The secretion produced no observable effects on the mantids when it was dropped into their mouths. They swallowed it and resumed their movement. After, they caught and ate other preys presented to them in a similar manner to that of the control not treated.

The subcutaneous injection of the secretion into the body of mice (*M.musculus*)and rats (*Rattus rattus*) resulted only in paralysis. Paralysis was manifested as the inability of the animals to move their limbs after they were injected. The subcutaneous injection of 0.1 and 0.5mm$^3$ of the secretion had no effect on the rats, however, the injection of the same amount of secretion into mice caused paralysis (Table 2).The recovery time was directly proportional to the volume of secretion injected into the mice and rats (Table 2).

TABLE 2

*Effect of infected repellent secretion of adult Z. variegatus on rat, Spraque dawley and albino mice, Mus musculus*

| Volume of secretion nfection (mm$^3$) | Rats (n=5) | Effect | Mean recovery time (mins+-S.E) |
|---|---|---|---|
| 0.1 | | No effect | - |
| 0.5 | | No effect | - |
| 1 | | Paralysis | 7 +- 1.07 |
| 3 | | Paralysis | 19 +- 1.40 |
| 5 | | Paralysis | 36 +- 1.59 |
| | Mice (n-5) | | |
| 0.1 | | Paralysis | 12 +- 1.26 |
| 0.5 | | Paralysis | 17 +- 1.14 |
| 1 | | Paralysis | 36 +- 1.40 |
| 3 | | Paralysis | 50 +- 2.14 |
| 5 | | Paralysis | 99 +- 3.28 |

Paralysis - inability to move limb
Recovery time - Time required for resumption of limb movements.

The experimental rats and mice used weighted between 100-120g and 35-50g respectively.

**Effect on the hind paw of rat:** The extent of the oedema produced in the hind paw of rats infected with carraegenin and the repellent secretion are given in Table 3. The oedema with a mixture of carraegenin and the repellent secretion was more pronounced than the oedema with 0.1mm3 portions of carraegenin and repellent secretion administered separately. The oedema resulting from caraegenin and the secretion separately showed that the peak of oedema was 6 hours after injection. (Table 3) while the oedema produced by the mixture of carraegenin and secretion peaked at the 3rd hour and persisted up to 6 hours.

**On the contraction of smooth muscle in guinea-pig ileum, and rat stomach and colon**: The muscle contraction induced by different molarities of acetycholine (Ach) was compared to that elicited by different dilution's of the secretion. The muscles of the guinea pig ileum and the rat stomach contracted in response to the secretion studied while that of the rat colon showed no response to the secretion (Table 4). The response of guinea-pig ileum and rat stomach to Ach at 8 x 10-8M was taken as maximum (100%), the amount of the other contractions are expressed as a percentage of it. The contractions obtained from the guinea pig ileum following the addition of the secretion at different were more pronounced compared to the rat stomach. The response of the guinea pig ileum to 0.4 dilution level of the secretion showed a 50% increase over that obtained at 0.2 dilution level. The same increased was observed for the contracion of rat stomach at 0.1 and 0.2 dilution levels on one and, 0.4 and 0.8 dilution levels of the secretion on another hand.

TABLE 3

*Oedema in the hind paw of wister rats, Spraque dawley induced by carragaenin and the repellent secretion of* Z. variegatus

| Time (hr) | Mean Diameter (mm) of oedema +-S.E. | | | | Differences in mean diameter (mm) | | | | % increase in diameter over initial diameter | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | C | C+S | D | S | C | C+S | D | S | C | C+S | D |
| 0 | 26.0+ -4.4 | 26.8+ -0.95 | 25.8+ -2.3 | 26.0+ -2.08 | - | - | - | - | - | - | - | - |
| 3 | 34.4+ -2.45 | 35.5+ -3.94 | 39.1+ -2.08 | 27.0+ 02.65 | 7.5+ -1.94 | 8.7+ -2.99 | 13.3+ -0.22 | 1+ -0.57 | 28 | 32.5 | 52.0 | 3.90 |
| 6 | 36.1+ -3.39 | 38.8 +-3.98 | 39+-1 +-2.89 | 26.7+ -2.89 | 9.2+ -1.00 | 11.5+ -3.03 | 13.3+ -0.59 | 0.7+ -0.8 | 34 | 43 | 52.0 | 2.70 |
| 12 | 31.6+ -1.51 | 34.5+ -5.67 | 36+ -3.51 | 26.0+ -3.61 | 4.7+ -2.88 | 7.2+ -4.72 | 10.2+ -1.21 | 0 | 17 | 29 | 39.5 | - |
| 24 | 28.4+ -2.28 | 28.6+ -4.09 | 34.3+ -2.08 | 26.0+ -3.61 | 1.5+ -2.12 | 1.8+ -3.14 | 8.5+ -0.22 | 0 | 5.6 | 6.7 | 32.9 | - |

(N=6)

S = 0.1 mm3 of the repellent secretion of adult Z. variegatus
C = 0.1 mm3 of carragaenin suspension (1w/v in 0.9% saline solution)
C+S = 0.05 mm3 of carragaenin + 0.05mm3 of the repellent secretion
D = 0.1 mm of distilled water

TABLE 4

*Smooth muscle contractions induced by the repellent secretion of* Z.variegatus *compared to that by acetylcholine*

| Ach concentration (M) | Size of Contractions | Ach obtained (mm) | Dilution factor of secretion | Secretion (s) Contraction obtained (mm) | |
|---|---|---|---|---|---|
| | GPI | RS | | GPI | RS |
| $10^{-8}$ | 23.3+-1.73 | 23+-2.0 | 0.1 | 10+-2.1 (14%) | 6+-0.45 (10.2%) |
| $2 \times 10^{-8}$ | 35.7+-0.89 | 23.3+-3.6 | 0.2 | 15+-1.96 (21) | 12+-1.8 (20.4%) |
| $4 \times 10^{-8}$ | 50.3+-3.2 | 33.7+-1.50 | 0.4 | 30+-2.3 (42%) | 15+-0.31 (25.4%) |
| $8 \times 10^{-8}$ | 71.7+-1.65 (100%) | 59+-2.4 (100%) | 0.8 | 40+-1.0 (56t%) | 30+-0.69 (50.8%) |

(n = 5)

Percentages in brackets are comparing with maximal contraction obtained (Ach concentration of $8 \times 10^{-8}$)

S = Repellent secretion of adult *Z. variegatus*
GPI = Guinea pig ileum
RS = Rat stomach

## DISCUSSION

The secretion of *Z. variegatus* was lethal or does it produced any permanent effect on laboratory animals (mice, rats) and other insects (ants, termites and praying mantids). These results differ from the effect produced by the secretions of *P. bufonis* (Orthoptera: Prygomorphidae) (Fishelson 1960) and *P. hieroglyphics* (Abushama 1972) which led to the death of the animals into which they were injected.

The secretion of *Z. variegatus* induced oedema in the hind paw of the rat similar to that induced by the known inflammatory agent carraegenin (Table 3) while the additive effects of the carraegenin and the secretion produced oedema that reached its peak earlier, in the 3rd hour, earlier than the two separately. The secretion also has similar pharmacological properties to that of *P. bufonis* (von Euw *et al* 1967) in its ability to induce contraction of smooth muscles. Although, the factor responsible for this pharmacological property of *Z. variegatus* secretion to induce contraction of the muscles needs to be identified, the susbtantial influx of $Ca^{2}$+ from extracellular medium is known to cause the contraction of smooth muscle (Bolton 1979) and since secretion of *Z. variegatus* is rich in $Ca^{2}$+ (Idowu & Modder 1998), it is possible to assume that it is probably one of the factor responsible for the contraction of smooth muscles.

The study showed that the odours of the whole body and the repellent secretion of *Z.variegatus* were clearly recognised by human beings. No significant difference exist in the perception of the odours. The volunteers described the odour as offensive, unpleasant and unattractive. These same description were used for the odour of related grasshoppers (Whitman 1990). The study has also shown that the degree of perception of the odour was highest in the 6th and adult instars. It is in these instars were recorded appreciable volume of secretion (Idowu 1996). Studies have also shown that the grasshopper is avoided by invertebrate and vertebrate predators because of its body odour (Idowu 1997).

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