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RESEARCH ARTICLE

EFFECT OF JUVENILE HORMONE ANALOGUE (FENOXYCARB) ON OVARIAN DEVELOPMENT OF DYSDERCUS SIMILIS

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ABSTRACT

Fenoxycarb, an analog of the juvenile hormone (JH) in insects, has been evaluated in vivo and in vitro on some pest. It exhibits potent insect juvenile hormone mimic activity and thus causes serious disturbances in the development, reproduction and behavior of a wide range of insects. This insect growth regulator (IGR) was applied topically on adult *Dysdercus similis*. The treatment induced an increase of vitellogenesis at low dose however, vitellogenesis, chorion formation were inhibited and atrophied of the oocyte was observed at high dose. A drastic decrease of carbohydrate, lipid and protein contents in ovaries. Thus, it seemed that females are sensitive to fenoxycarb.

INTRODUCTION

Juvenile hormones are important regulators of insect development and diapause (Bowers, 1982). In adults, they are the key hormones involved in reproduction (Riddiford, 1985). The main types of insect growth regulators used commercially are Juvenile hormone and chitin synthesis inhibitors (Parrella and Murphy, 1998).

Pesticide development has produced some chemicals with specific modes of action for controlling pests, including the group of juvenile hormone analogs (Dhadialla *et al.,* 1998). The juvenile hormone analogs interact with the natural hormones of insect development (Slama,1995) to inhibit metamorphosis to adult stage (Miyamoto et al., 1993).

Fenoxycarb, exhibits potent insect juvenile hormone mimic activity and thus causes serious disturbances in the development, reproduction and behavior of a wide range of insects (Dorn et al., 1981: Okot-Kotber *et al*., 1991, Grenier, S. *et al.,* 1993; Schneider, M. *et al.,* 1995; Ujvary, I *et al*., 1996). It is used for insect control in aquaculture, forestry and stored products and is also employed as a public health insecticide (Edwards, J.P. *et al.,* 1991 and Evan R.G. *et al.*, 1995).

It is reported to have an ovicidal activity (Charmillot *et al*., 2001; Bortolotti *et al*., 2000; Kayser *et al.,* 2001). The aim of the present study is to examine the histopathological changes occurred in the ovaries of the adult female *Dysdercus similis.*

MATERIAL AND METHODS

Dysdercus similis nymphs were collected from lady finger fields near Sagar (M.P), India. They were reared in the laboratory in glass-fronted cages and were fed regularly on moist cotton seeds to prevent starvation. RH-34343 (Fenoxycarb) a juvenile hormone analogue from Sigma, was used as a test substance. The compound was dissolved in acetone (1 µl per insect) and topically administered at two doses: (0.01 and 0.03 µg/insect, corresponding respectively to LD50 and LD90).

Histopathological studies

The ovaries of control females and females treated 2, 4, 6 and 8 days were fixed in Cornoy's fluid (6:3:1); paraffin blocks were made in usual way were sectioned at 6µm. Delafield's haematoxylin and eosin staining technique (Pear, 1960) was used for histopathological studies.

Biochemical composition of gonads

Fenoxycarb was topically applied at two doses (0.01 and 0.03 µg/insect) on newly emerged adult females. Ovaries of newly emerged adults were removed and were then homogenised in trichloroacetic acid (20%, w/v) and carbohydrate, lipid and protein of ovaries were extracted according to Shibko et al. (1966) and evaluated according to Duchateau and Florkin (1959), Goldsworthy et al. (1972) and Bradford (1976), respectively.

RESULT AND DISCUSSION

Ovarian lipid, carbohydrate and protein concentrations

Obtained results showed that fanoxycarb treatment caused a significant decrease on carbohydrate ($P<0.05$) and lipid ($P<0.01$) concentrations in ovaries (Figure 1) with a dose-response relationship. On the other hand, only protein content in ovaries was decreased ($P<0.01$) compared to control.

In *Dysdercus similis* the ovaries are of the typical acrotrophic type and occupy most of the abdominal region. The female treated topically with 0.01 and 0.03 µg/insect of 1 µl acetone.

Figure 1. Effect of fenoxycarb, (LD50 and LD90) on ovarian concentrations of carbohydrates, lipids and proteins (µg/mg of ovary) of females of *Dysdercus similis* (mean ± SD; n = 3; Different from control *$P<0.05$; **$P<0.01$).

Adverse effect on the developing ovaries was found after treating with this doses.

In the control females, the privitellogenic and vitellogenic phase were normal. The oocytes were wrapped in distinct, regular follicular epithelium with a sufficient amount of yolk.

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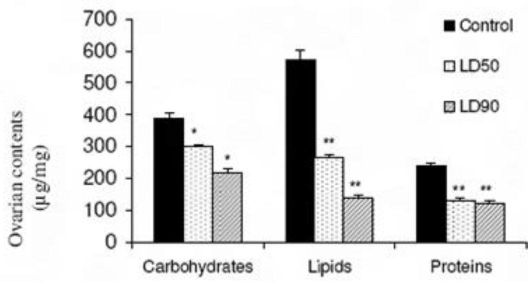
The tunica properia had a regular outline and was firmly attached to the follicular epithelium.

Figure 1



The progressive changes which took place in females treated from 2 nd to 8 th days with different doses were as follows.

From the present observations, it is observed that the egg laying was took place early as compared to the control. After treatment with 0.01 and 0.03 µg/insect fenoxycarb the two days ovary were dull in appearance, but the ovaries were morphologically fairly normal and pathological oocytes was less distinct than in the ovaries of females treated for eight days. The follicular cells are suffering from degeneration and vacuolization. Follicular cells apparently regulate transfer of materials for oocyte growth. Histochemical studies on the telotrophic ovarioles of *Crynodes pergrinum* showed that follicle cells synthesize RNA which is exported to oocytes during its early stages of growth (Ram and Ramamurty, 1979).

The topical application of fenoxycarb (test compound) to adult insects of California fivespined ips, *Ips paraconfusus*, reduced both egg production by females and egg hatching as reported by Chen and Borden, (1989). Bhargava and Urs, (1993) reported mortality effect on the eggs of rice moth *Corcyra cephalonica* exposed to various doses of hydroprene. It has been reported that Juvenile hormone analogues are more effective at the beginning stage of metamorphosis and embryogenesis in insect such as freshly ecdysed last larval instars, freshly ecdysed pupal instars, and freshly deposited eggs (Dhadialla *et al.,* 1998). Thus embryogenesis is disrupted when young eggs are treated with JHAs. Eggs exposed to fenoxycarb and other juvenile hormone analogs showed disruption of the blastoderm with associated cellular and organellei disruption (Dhadialla *et al.,* 1998).

Application of low doses of juvenile hormone to adult workers *Apis indica* led to an increase in vitellogenin levels, whereas high doses of this hormone or its synthetic analogues, inhibited this response (Rutz *et al.*, 1976; Engels *et al*., 1990; Pinto *et al*., 2000).

In the adult females of Hemimetabola (Dictyoptera to Hemiptera) and Coleoptera, JH is the main regulator and pleiotropically controls most aspects of female reproduction.

The major role of JH in reproduction is to regulate vitellogenin (Vg) gene expression in the fat body, generally and in ovarian follicular epithelium (Engelmann, 1983, 2003; Wyatt and Davey, 1996; Belles, 2004). In the oviparous *Periplaneta americana* (Blattidae), Weaver and Edwards (1990) have shown that allatectomy or treatment with inhibitors of JH synthesis blocks Vg production, oocyte growth, and ootheca formation, whereas JH treatment restores these processes. The effective doses to induce vitellogenesis in females in vivo range from 1µg of JH I to 25µg of JH III in vivo. Methoprene, a potent JH analog (JHA), induces vitellogenesis in adult males as well (Dorn- Wheeler and Engelmann, 1997). Induction of Vg synthesis in female *L. migratoria* by JH homologs is only achieved with repeated doses or with high doses co-injected with a JH esterase inhibitor (Wyatt *et al*., 1987). Whereas synthetic JHAs doses between 2 and 30µg have a much more potent vitellogenic action (Edwards *et, al*., 1993; Zhang *et al.,* 1993). For Hemiptera, the CA were shown to be necessary for vitellogenesis in *Rhodnius prolixus* by Wigglesworth in the 1930s and this observation has since been confirmed for several other hemipteran species (Wyatt and Davey, 1996, 1997; Belles, 2004) Later studies demonstrated that allatectomy of *R. prolixus* does not totally abolish Vg synthesis, but JH treatment does restore normal production (Wang and Davey, 1993). *Oncopeltus fasciatus* chemically allatectomized with precocenes produced the Vg precursor, but its conversion to mature Vg, which is incorporated into the oocytes, did not takeplace, as shown with electrophoresis of native proteins and immunodiffusion (Kelly and Hunt, 1982). Precocene treatment of aphid nymphs inhibited oocyte development in embryos inside the parental ovaries, whereas JH reversed this inhibition (Hardie, 1987).

Topical application of JH also increased vitellogenin levels in flies (Aui *et al.*, 1981). *In P. pictus* and *Dysdercus* the JH no doubt activated the vitellogenin synthesis but an over dose of JH-controls the regulation of CA through the neurosecretory cells. Tobe (1981) suggested that low doses of JH initiate the synthesis and high titer inhibits the synthesis in CA. In *P. pictus* and *Dysdercus* the administered doses (0.01% and 0.03%) appear to be high and may have inhibited the activation of CA but it appears that this inhibition is through the NS cells as reported by Verma, (1990).

The results of present study showed arrested development of ovary leading to its necrosis by fenoxycarb is similar to those as reported by Verma(1990). Degeneration of follicular epithelium layer as described above would present. It from functioning properly and thus, uptake of nutrients from haemolymph would be affected. Degeneration of follicular epithelium bridges between oocytes has also been observed by Banerjee and Sahai (1987). Perhaps this leads to dependence of oocytes on one another for nutrition and protection when follicular epithelium investing them fails to function properly.

CONCLUSION

Appearance of vacuoles and progressive formation of large spaces and absence of yolk granules in fenoxycarb treated ovaries indicated gradual absorption of oocytes, steadily increase vacuolization of ooplasm. Owing to degeneration of yolk and to deformity and subsequent loss of follicular epithelium during the first reproductive cycle indicated that the damage of female gonad of *Dysdercus*. Fenoxycarb treatment is irreparable and leads to its sterility. The cytotoxic effects observed in ovaries of females led to interference with egg maturation. As part of the severe histopathological effect, almost the entire contents of the ooplasm were resorbed. In the present investigation it was observed that the yolk deposition was less as compared to control ovary and also significantly reduces reproductive potential of adults, in particular by falling fertility, fecundity and biochemical composition of female gonads.

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