Original Research Article

Biochemical effects of two phytochemical mixture Eugenol with (+)-O-Methylarmepavine during the morphogenetic development of Blattella germanica

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Abstract

The German cockroach, Blattella germanica, is an important sanitary pest. It is cosmopolitan and abundant in homes and other human installations. Besides contaminating the places they live in with their excrements and exuviae, they are the mechanical vectors for a number of human pathogenic microorganisms and also produce allergens. The food items are contaminated by the in and around movements of German cockroaches which become unsuitable for the human consumption. Proteins are the characteristic components of the tissues which play a major role in morphogenetic events. The quantitative estimation of the proteins in the fatbody, haemolymph and ovary of nymphs and the adult was carried out to show their interrelationship. Anti gonadotropic action of Eugenol with (+)-O-Methylarmepavine on Blatella germanica was studied in the fatbody, haemolymph and the ovarian proteins during the morphogenetic development.

Key Words: (+)-O-Methylarmepavine, Eugenol, Blattella germanica, fatbody, haemolymph and ovary.

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INTRODUCTION

The German cockroach, *Blattella germanica*, is an important sanitary pest. It is cosmopolitan and abundant in homes and other human installations (Cochran, 2003). The German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), is by far the most serious and predominant cosmopolitan pest in the world due to changes in human travel, commerce, and the urban environment (Milner RJ *et al*., 2007). The complete life cycle of the cockroach is roughly 100 to 200 days for females, during which 10,000 descendants of a single cockroach can be produced. German cockroaches carry organisms that cause disease, principally bacteria, protozoans, and viruses that cause gut symptoms like food poisoning, dysentery, and diarrhea. They also produce malodorous secretions that taint the flavor of food, and their cast skins are allergens, but they are mostly aesthetic pests. Their flat bodies allow them to live in cracks and crevices of human habitations, often in large numbers. German cockroaches scavenge on any food, and even non-food stuffs such as soap and glue that are left around and are commonly found in garbage receptacles (Jacobs, 2007; Kunkel, 2008; McCandless, 2005). These Cockroaches can readily move from contaminated zones and garbage and create the opportunity to spread disease-causing organisms on food and food preparation surfaces(Bennett GW.2008) Many studies have highlighted a possible and potential risk of human contamination through bacteria carried by cockroaches in connection with human habitats(Oliva

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GR,2010, Jeffery J,2012.) Proteins are the characteristic components of the tissues. Proteins play a vital role in the insect development and morphogenetic events. Proteins also play an important role in the production of certain enzymes and hormones, which control many chemical processes necessary for the metabolism. The fat-body synthesizes the proteins during the larval development and the proteins predominantly include the storage proteins (Wigglesworth, 1972). The proteins which are available in the immature stages in the fat body are used as a source of amino acid pools for organogenesis during the metamorphosis (Raja *et al*., 1986). The haemolymph is the single extra cellular fluid of the insects which communicates with the organs and the tissues. it is the reservoir for many materials essential for a variety of insect processes. Thus the changes in the composition of haemolymph reflect the morphogenetic changes and related biochemical transformations taking place in the insect tissue (Cohen.2010 and Sugumaran, 2010). Proteins provide the chief structural elements for different tissues and in turn are controlled by hormones. Hence an attempt was made to study the combined effect of Eugenol with (+)-O-Methylarmepavine on the protein content in the fat body, haemolymph and ovaries during the morphogenetic development of *Blattella germanica* .

MATERIAL AND METHODS

Test Insect: German cockroaches are one of the most common cockroach species found in households. German cockroaches undergo three distinct life phases, egg, nymph and adult. Their entire life cycle spans approximately 100 days, although this is dependent on factors such as temperature and diet. German cockroaches have 6 nymphal stages occurring over a period of 6 to 31 weeks. They express incomplete metamorphosis. zygotes develop within eggs and hatch directly into nymphs, which then grow into adult cockroaches. The pest *Blattella germanica* is reared on carbohydrate and gram diet in a three chambered containers at the temperature of 25 ± 1°C and RH 65 ± 5 for the experimental purpose .

Test Solution: Equal amount of Eugenol and (+)-O-Methylarmepavine mixed and taken as test solution. The test compounds (+)-O- Methylarmepavine extract from the leaf of Annona squamosa Procured from the Natural chemistry lab, Department of chemistry, Osmania University and Eugenol procured in the form of clove oil. The freshly moulted 4th, 5 th and 6 th instar nymphs were topically treated on the abdominal region with 2µg /µl of test solution (Eugenol with (+)-O- Methylarmepavine) of acetone / larva. Controls were treated with only acetone. 40 larvae were treated each time and the experiment was replicated five times. Haemolymph was collected using the rapid centrifugation method of Nation and Thomas (1965) Phenyl thio urea was added to the haemolymph to inhibit the tyrosinase activity. Haemolymph was centrifuged at 2500 rpm to remove haemocytes. The fat body and ovaries of control and treated resultants were dissected in freshly prepared Ringer's solution. The proteins were extracted from these tissues and the protein was estimated by Lowry *et al*., (1951) method.

RESULTS

Table 1: Protein content in the Fat body of Blattella germanica in control and treated (Eugenol with (+)-O-Methylarmepavine solution) resultant insects

Stage	Age in days	Control	Treated by Eugenol with (+)-O-methylarmepavine solution
IV Instar Nymph	Day1	0.642±0.0007	0.628±0.0008 ^{NS}
	Day7	0.677±0.0006	0.627±0.0002
	Day15	0.752±0.0005	0.674±0.0009
V Instar Nymph	Day1	1.873±0.0009	0.854±0.0008
	Day7	2.436±0.0007	0.918±0.0007
	Day15	2.834±0.0009	0.982±0.0008
VI Instar Nymph	Day1	3.126±0.0005	0.843±0.0007
	Day7	2.681±0.0008	0.724±0.0006
	Day15	1.683±0.0009	0.621±0.0008
Adult *B.germanica*	Day1	1.484±0.0007	0.543±0.0009
	Day7	1.185±0.0009	0.323±0.0005
	Day15	0.851±0.0006	0.244±0.0007

The values are expressed in mg of protein /gm of fat body. Each value is the mean ± Standard error of Six individual observations. The difference between control and treated is statistically significant (P>5%). ^{NS} denotes not significant (P<5%).

Table 2: Protein content in the Haemolymph of *Blattella germanica* in control and treated (*Eugenol with (+)-O-Methylarmepavine solution*) resultant insects.

Stage	Age in days	Control	Treated by Eugenol with (+)-O-methylarmepavine solution
IV Instar Nymph	Day1	0.834±0.0006	0.816±0.0005 ^{NS}
	Day7	0.982±0.0006	0.884±0.0006
	Day15	1.643±0.0008	0.921±0.0006
V Instar Nymph	Day1	2.643±0.0009	0.976±0.0008
	Day7	3.414±0.0007	0.998±0.0009
	Day15	3.726±0.0006	1.012±0.0008
VI Instar Nymph	Day1	3.624±0.0007	1.243±0.0002
	Day7	3.146±0.0008	0.817±0.0005
	Day15	2.863±0.0009	0.714±0.0008
Adult *B.germanica*	Day1	1.241±0.0007	0.623±0.0006
	Day7	0.983±0.0005	0.514±0.0006
	Day15	0.425±0.0007	0.214±0.0008

The values are expressed in mg of protein /gm of Haemolymph. Each value is the mean ± Standard error of Six individual observations. The difference between control and treated is statistically significant (P>5%). NS denotes not significant (P<5%).

Table 3: Protein content in the Ovaries of *Blattella germanica* in control and treated (*Eugenol with (+)-O-Methylarmepavine solution*) resultant insects.

Stage	Age in days	Control	Treated by Eugenol with (+)-O-Methylarmepavine solution
VI InstarNymph	Day1	0.726±0.0007	0.712±0.0005 NS
	Day7	0.943±0.0008	0.793±0.0006
	Day15	1.743±0.0009	0.918±0.0007
Adult *B.germanica*	Day1	3.426±0.0008	1.123±0.0009
	Day7	2.181±0.0007	0.743±0.0006
	Day15	1.562±0.0005	0.248±0.0007

The values are expressed in mg of protein/gm. Each value is the mean ± Standard error of Six individual observations. The difference between control and treated is statistically significant (P>5%). NS denotes not significant (P<5%). Estimation of protein content in the fat body, haemolymph and ovaries of the Eugenol with (+)-O-Methylarmepavine solution treated resultant nymphs and adult

Estimation of fat body protein: The protein content in the fat body of different stages of life cycle of *Blattella germanica* treated with test solution (Eugenol with (+)-O-Methylarmepavine) exhibits a remarkable difference when compared with the controls.

Nymphal Stage: The protein content in the fat body on the 1st day of IV instar nymph was 0.642 ± 0.0007 mg/gm in control insect and it is 0.628 ± 0.0008 was not significant in the treated insect. On 15th day control has 0.752 ± 0.0005 was significantly differ with treated insects recorded as 0.674 ± 0.0009. In V instar nymph, on the 1st day in controls was 1.873 ± 0.0009 and on 15th day 2.834 ± 0.0009 whereas in case of treated insects significant difference recorded as 0.854 ± 0.0008 on 1st day to 0.982 ± 0.0008 on 15th day. In VI instar nymph, on the 1st day in controls was 3.126 ± 0.0005 and on 15th day 1.683 ± 0.0009 whereas in case of treated insects significant difference recorded as 0.843 ± 0.0007 on 1st day to 0.621 ± 0.0008 on 15th day.

Adult Stage: In adult, on the 1st day in controls was 1.484 ± 0.0007 and on 15th day 0.851 ± 0.0006 whereas in case of treated insects significant difference recorded as 0.543 ± 0.0009 on 1st day to 0.241 ± 0.0007 on 15th day. (Table – 1)

Estimation of protein content in the haemolymph: The protein content in the haemolymph of different stages of life cycle of *Blattella germanica* treated with test solution(Eugenol with (+)-O-Methylarmepavine) exhibits a prominent variation when compared with the controls.

Nymphal Stage: The protein content in the fat body on the 1st day of IV instar nymph was 0.834 ± 0.0006 mg/gm in control insect and it is 0.816 ± 0.0005 was not significant in the treated insect. On 15th day control has 1.643 ± 0.0008 was significantly differ with treated insects recorded as 0.921 ± 0.0006. In V instar nymph, on the 1st day in controls was 2.643 ± 0.0009 and on 15th day 3.726 ± 0.0006 whereas in case of treated insects significant difference recorded as 0.976 ± 0.0008 on 1st day to 1.012 ± 0.0008 on 15th day. In VI instar nymph, on the 1st day in controls was 3.624 ± 0.0007 and on 15th day 2.863 ± 0.0009 whereas in case of treated insects significant difference recorded as 1.243 ± 0.0002 on 1st day to 0.714 ± 0.0008 on 15th day. In **Adult Stage** : In adult, on the 1st day in controls was 1.241 ± 0.0007 and on 15th day 0.425 ± 0.0007 whereas in case of treated insects significant difference recorded as 0.623 ± 0.0006 on 1st day to 0.214 ± 0.0007 on 15th day. (Table – 2)

Estimation of protein content in the ovaries: The protein content in the ovaries of different stages of life cycle of *Blattella germanica* treated with test solution (Eugenol with (+)-O-Methylarmepavine) exhibits a prominent variation when compared with the controls. The protein content in the ovaries on the 1st day of VI instar nymph was 0.726 ± 0.0007 mg/gm in control insect and it is 0.712 ± 0.0005 was not significant in the treated insect. On 15th day control has 1.743 ± 0.0009 was significantly differ with treated insects recorded as 0.918 ± 0.0007. (Table – 3) In adult, on the 1st day in controls was 3.426 ± 0.0008 and on 15th day 1.562 ± 0.0005 whereas in case of treated insects significant difference recorded as 1.123 ± 0.0009 on 1st day to 0.248 ± 0.0007 on 15th day.(Table– 3)

DISCUSSION

Proteins are the characteristic components of the tissues. Proteins play a vital role in the insect development and morphogenetic events. Insect haemolymph is in direct contact with the tissues and any disturbances in the insect body are reflected by changes in the protein level and protein pattern of the haemolymph. It is influenced by the complex relations of the metabolism, synthesis and uptake of protein by the fat body tissue. (Raja *et al*., 1986). Eugenol with (+)-O-Methylarmepavine solution treated resultants exhibited a decline in the protein content when compared with the control nymphs. In control insects, protein content in the ovaries rapidly rise from the 1st day of IV instar 15 th day of the V instar nymph in fat body and haemolymph. Due to the morphological transformation the protein level gradually

increases during this period. The protein level and protein pattern of the haemolymph is influenced by the complex relations of the metabolism, synthesis and uptake of proteins by the body tissues of the insect. This was declined gradually in VI instar nymphs due to the preparation oogenesis in adults. The fat body diminishes its activity in intermediary metabolism of protein synthesis and changes to function chiefly in storing nutrients for adult development. At this stage the fat body protein concentration greatly increases. This results are in conformity with that of DeenaVardhini, (1997) . A decline in fat body concentration and a concomitant rise in the protein concentration in ovaries is observed in the later (Kilby 1963). This is correlated to vitellogenesis and to the possibility that the excess proteins of the fat body are utilized by the growing oocytes, confirming the results of (Raja *et al*., 1988 ;Anitha *et al*., 2000). The synthesis of proteins in the fat body and its transport by haemolymph and uptake by the oocytes is the main factor on which the vitellogenesis of the insect depends. (Miller *et.al*.,1982).

CONCLUSION

Eugenol with (+)-O-Methylarmepavine solution is responsible for the decline in the protein concentration in various tissues at different stages of the treated resultant *Blattella germanica* and it might be influencing the hormonal activity in treated resultants. The treatment of the Eugenol with (+)-O-Methylarmepavine solution influences protein synthesis, Storage and uptake of proteins by the fat body, Haemolymph and ovaries resulting in formation of abnormal adults which shows low fecundity thus suppressing the population of *Blattella germanica*.

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