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ANTIGENOTOXIC EFFECTS OF ROYAL JELLY IN THE SEX LINKED RECESSIVE LETHAL TEST WITH *DROSOPHILA MELANOGASTER*

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The antitoxic and antimutagenic effect of royal jelly was assayed by a standard testing procedure on Drosophila melanogaster. The similarity of metabolic pathways between Drosophila and mammals makes the test widely applicable for detecting the impact of various potential promutagens and antimutagenic effects could be recognized accordingly. The flies were treated with the potent mutagen MMS, alone and combined with royal jelly. The frequency of sterile males and sex linked recessive lethal mutations increased significantly after MMS treatment and decreased after combined treatment. The results strongly indicate that, in addition to its well documented action on development, life-span and reproductive ability, royal jelly has an antimutagenic potential as well.

Key words: antigenotoxicity, Drosophila melanogaster, mutagenicity, royal jelly, sterility

INTRODUCTION

While detecting genotoxicity of compounds and mixtures in food and environment have called for constant surveys in pharmacy and biology for a long time, the antimutagenic and antitoxic effects of certain compounds and mixtures raised attention only recently (Simic *et al*., 1998; Weisburger, 2001).

An array of standard *in vitro* and *in vivo* genotoxicity test systems has been established on different organisms (bacteria, yeast, *Drosophila*, mammals, etc.) (Venitt and Parry, 1984). Antigenotoxic and antimutagenic effects of substances are possible to test using the same test protocols as for genotoxicity testing. This can reveal the capability of substances in preventing the damage or performing corrections on damaged genetic material. The fruit fly *Drosophila melanogaster* has been used in a number of studies on genotoxicity, as well as antigenotoxicity, of various compounds, through tests developed for the detection of somatic or germinative mutations (Fujie and Fujikawa, 1996).

Royal jelly is an extremely nutritious product of worker bees, fed to larvae which become queen bees. It accounts for their significant increase in size, longevity and fertility. Royal jelly is mainly composed of carbohydrates, proteins and lipids. It is rich in pheromones, almost all amino acids, B group vitamins and

vitamin A, C, D and E, aspartic acid, gelatine, sugars, sterols, phosphorous compounds, essential fatty acids, acetylcholine, nucleic acids, and numerous trace ingredients, which are all important in royal jelly's documented therapeutic and nutritional properties. While immunological and physiological benefits from royal jelly are well documented, its antigenotoxic potential is still unknown.

Aiming to detect mutations in the male germline stages and the effect of royal jelly on their frequency, the standard test for detecting sex linked recessive lethal mutations on *Drosophila melanogaster* was used in this study. The test is based on the characteristics of sex-linked inheritance, and detects gene mutations, small deletions, or certain types of chromosome aberrations, which are lethal in hemizygous and homozygous conditions before the adult stage (Wurgler and Graf, 1985).

The estimated total number of loci on the X chromosome that can mutate to recessive lethals is up to 800, which make the test results evaluation reliable. The similarity of metabolic pathways between *Drosophila* and mammals and it's ability to activate promutagens makes the results of this test widely applicable.

MATERIAL AND METHODS

The standard procedure for the detection of sex linked recessive lethal mutations (SLRL test) on *Drosophila melanogaster* was applied (Mitchell and Combes, 1984; Wurgler and Graf, 1985). The test procedure detects possible mutations in the second generation of the progeny of treated males. The treated males are crossed individually with virgin females from *B a s c* stock, with visible genetic markers on the X chromosome. The sterility is assessed from this cross as the number of vials without larvae. The first generation of the progeny is allowed to intercross. The frequency of sex linked recessive lethal mutations is detected by examining the flies in the second generation. All wild type males in the second generation, possess the same treated X chromosome in a hemizygous condition. Any recessive lethal mutation is expressed before the imago stage. Thus, the absence of wild type males in the second generation indicates the occurrence of such mutations.

An important feature of this test is that treated males are successively mated with marker stock females (broods I, II and III), so the effect of substances on sterility and frequency of mutations is assayed at each of the germ-line stages: spermatozoa-spermatids-spermatocites.

For testing the effect of royal jelly in this study, its saturated solution (5%) in 1% sucrose (negative control and solvent in all treatments) is used. As a strong mutagen, the 0.00125 M solution of methyl-methane-sulphonate (MMS) was fed to 2 days old *D. melanogaster* males from the long-time established *Canton-S* laboratory stock, which ensures that X chromosomes of the males used for testing are free from pre-existing lethals.

The combined treatment was performed by feeding flies successively with royal jelly - MMS - royal jelly in 3 x 24 hour periods.

The test was performed under standard laboratory conditions for *Drosophila melanogaster*: 25°C and humidity about 60%.

RESULTS

Table 1. gives the percent of sterile *D. melanogaster* males after all treatments, in three broods. The results are analyzed by the Z-test for difference in proportions (Zar, 1984).While royal jelly (RJ) does not significantly influence the male sterility in any of the three broods, MMS increases it significantly in each (Z_I=6,132; Z_{II}=2,148; Z_{III}=6,264. A combined treatment (RJ-MMS-RJ) reveals that royal jelly decreases the sterility effect of MMS with high significance in the first and the third brood (Z_I=6,330 and Z_{III}=2,673).

Table 1. Frequencies of sterile *Drosophila melanogaster* males in three broods after treatments with sucrose (negative control), royal jelly (RJ), methyl-methane-sulphonate (MMS) and combined treatments of MMS and royal jelly (RJ-MMS-RJ). Asterix indicates significantly higher frequency compared to control

Broods		Treatment			
		sucrose	RJ	MMS	combined
I	No of crossess	50	50	150	149
	No of sterile males	2	3	45	14
	frequency of sterile (%)	4.00	6.00	30.00*	9.39*
II	No of crossess	50	48	149	147
	No of sterile males	3	2	19	24
	frequency of sterile (%)	6.00	4.17	12.75*	16.32*
III	No of crossess	50	46	147	50
	No of sterile males	4	6	22	3
	frequency of sterile (%)	8.00	13.04*	14.96*	6.00
I+II+III	No of crossess	150	144	446	347
	No of sterile males	9	11	86	41
	frequency of sterile (%)	6.00	7.64	19.28*	11.81*

Table 2. gives the frequency of sex linked recessive lethal mutations occurring in *D. melanogaster* males under all treatments. The significance of differences are analyzed by a test for determining the statistical significance of mutation frequencies (Kastenbaum and Bowman, 1970). Methyl-methane-sulphonate significantly increases the frequency of sex-linked recessive lethal mutations in *D. melanogaster* males in all three broods (p_I=0,567, p_{III}=0,540, for α=0,01 and p_{II} =0,533, for α=0,05).

Table 2. Frequencies of sex linked recessive lethal mutations in the *Drosophila melanogaster* test after treatments with sucrose (negative control), royal jelly (RJ), methyl-methane-sulphonate (MMS) and the combined treatments of MMS and royal jelly (RJ-MMS-RJ). Asterix indicates significantly higher frequency compared to control

Broods		Treatment			
		sucrose	RJ	MMS	combined
I	No of chromosomes	291	287	222	250
	No of lethals	0	0	22	26
	frequency of lethals (%)	0.00	0.00	9.91*	10.40*
II	No of chromosomes	283	280	248	240
	No of lethals	0	0	32	11
	frequency of lethals (%)	0.00	0.00	12.90*	4.51*
III	No of chromosomes	286	284	244	285
	No of lethals	1	1	13	15
	frequency of lethals (%)	0.35	0.35	5.33*	5.26*
I+II+III	No of chromosomes	860	851	714	779
	No of lethals	1	1	67	52
	frequency of lethals (%)	0.116	0.118	9.384*	6.675*

The combined treatment of MMS and RJ yields a statistically significant decrease in mutations in the second brood and in all broods taken as a total (p_I=0,504 and $p_{I+II+III}$=0,478, for α=0,01).

DISCUSSION

Research data indicate that royal jelly increases the life span and fertility in many species, including humans. It also has a strong bactericidal and healing effect, stimulates the immune system and may produce anticancer effects (Broadhurst, 1999). In general, the wide array of its beneficial effects on health is due to the significant action of its particular compounds. Some authors report that 10-HDA (10-hydroxy-decanoic acid) is the most important in the anticancer activity, but pantothenic acid, found in high concentrations in RJ is an antioxidant and has protective effects as well. Thus, the beneficial effect of royal jelly may be due to a complex action of several substances.

The antigenotoxic effect of different compounds has been tested in *Drosophila*, mainly using somatic mutation tests (Rizki *et al*., 2001; Nakano *et al*., 1994). The sex linked recessive lethal test of genotoxicity has been chosen for the purpose in this paper, from a variety of tests in *D. melanogaster*, because of the known effect of royal jelly on fertility. The SLRL test provides the information of the

effect of the agents on sterility at three *Drosophila* male germ-line stages. The strongest effect of decreased sterility under RJ was obtained at the stage of spermatids and spermatogonia.

Mutagens vary widely in their potency for inducing mutations, thus is the sensitivity in detecting their mutagenic effects equally variable. It is expected that the antimutagenic effect will vary accordingly. The germ cell stages differ in sensitivity to potential mutagens and promutagens (Wurgler and Graf, 1985). According to the SLRL test procedure, cells were exposed in successive spermatogenetic stages (three broods): the first brood gives the effect of treated postmeiotic stages, spermatozoa and spermatids, the second brood on meiotic spermatocytes, and the third brood reveals the effect of the tested agents on the premeiotic stage (spermatogonia). According to literature data, toxic effects should be the most severe at this stage. The results obtained in the present paper show that spermatocytes are most sensitive to mutagenic activity of MMS, and that the strongest antimutagenic effect of royal jelly is expressed at this stage.

Despite the significant antitumor effect of royal jelly (Tamura *et al*., 1987), some studies of the possible antimutagenic activity of different apiculture products did not show significant protective abilities of royal jelly (Bariliak *et al*., 1996). The reason could be in the chosen test and experimental designs. The antimutagenic activity of a particular substance certainly depends on test conditions, organism used, developmental stage that is treated, etc. If larvae were fed carcinogens together with some potential antimutagen, the appropriate somatic mutation test would reveal its effects. However, the main action potential of royal jelly seems to be on germinative cells, so the SLRL test used here seems to be most suitable for detecting either antigenotoxic ot biogantigenotoxic effects.

The similarity of the metabolic pathways between *Drosophila* and mammals makes the SLRL widely applicable for detecting the impact of various potential mutagens and promutagens (Vogel, 1984). The results presented here indicate a protective role of royal jelly under the action of a strong mutagen such as MMS, at germ-line stages of *D. melanogaster*. Further studies in chronic and acute pre-treatment, as well as cotreatments of royal jelly with different mutagens and promutagens, in several test systems, are needed to provide the complete analysis of its antigenotoxic effect.

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ANTIGENOTOKSIČNI EFEKTI PČELINJEG MLEČA U RECESIVNOM LETALNOM TESTU POMOĆU *DROSOPHILA MELANOGASTER*

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SADRŽAJ

Antigenotoksični i antimutageni efekat pčelinjeg mleča ispitivan je korišćenjem standardne procedure testiranja pomoću *Drosophilae melanogaster*. Sličnost metaboličkih puteva izmedju *Drosophila* i sisara čini ovaj test široko primenljivim u otkrivanju uticaja različitih potencijalnih mutagena i promutagena, a analogno tome se može ispitivati i mogući antimutageni potencijal substanci. Mušice odgovarajućeg genotipa tretirane su jakim mutagenom, metil-metansulfonatom (MMS), samostalno i u kombinaciji sa mlečom. Učestalost sterilnih mužjaka i polno vezanih recesivnih letalnih mutacija rasla je značajno nakon tretmana sa MMS i opadala nakom kombinovanog tretmana. Dobijeni rezultati značajno ukazuju da uz već potvrđeni uticaj na razviće, dužinu života i reproduktivnu sposobnost, mleč ima i antimutageni potencijal.