EVALUATION OF *IN VIVO* GENOTOXICITY OF FORSKOLIN BY THE COMET ASSAY

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Introduction

Forskolin, found in the root of the plant *Coleus forskohlii* (Willd.) Briq. (Lamiaceae), has been consumed due to reports of its therapeutic properties, including use as a supplement for reducing body weight. Since there is little available information on the genotoxicity and/or antigenotoxicity of forskolin, the present study was aimed to assess the *in vivo* genotoxic activity of forskolin in different concentrations (0.5, 1, and 2 mg/mL of standard food) in somatic cells of third instar larvae of *Drosophila melanogaster* using comet assay.

Materials and methods

Larvae of wild type strain of *D. melanogaster* (Canton S, available from Bloomington Stock Center, Indiana, USA) were cultured at 25°C, 60% humidity and a 12:12 h light/dark regime on standard corn medium containing agar, sugar and yeast.

Three different concentrations (0.5, 1, and 2 mg/mL of standard food for Drosophila) of forskolin were evaluated to determine the genotoxic effect *in vivo*. Ethyl methanesulphonate (1 mM in PBS) was used as a positive control. The comet assay was performed according to Singh et al. (1988) with minor modifications as described by Mukhopadhyay et al. (2004). Immediately before use slides were stained with 80 mL of ethidium bromide (20 mg/mL). The images were visualized and captured with 40 objective lens of fluorescence microscope Nikon (Ti-Eclipse) attached to CCD camera.

TABLE 1. *IN VIVO* GENOTOXIC EFFECT OF FORSKOLIN BY THE COMET ASSAY

	Comet class					Total score[a]
	0	1	2	3	4	
NC[b]	78.6±0.9	21.4±0.2	0.00±0.00	0.00±0.00	0.00±0.00	21.4±0.4†
EMS[c]	55.5±0.71	5.6±0.34	11.1±0.61	5.6±0.54	22.2±0.8	133.4±1.2*
For 0.5[d]	76.2±0.22	23.8±0.54	0.00±0.00	0.00±0.00	0.00±0.00	23.8±0.15†
For 1[e]	74.8±0.30	25.2±0.23	0.00±0.00	0.00±0.00	0.00±0.00	25.2±0.10*†
For 2[f]	71.2±0.24	28.8±0.52	0.00±0.00	0.00±0.00	0.00±0.00	28.8±0.1*†

[a]Values represented mean±SEM from three independent experiments.

[b]NC; negative control group; [c]EMS; ethyl methanesulfonate, 1 mM.

[d]For 0.5, forskolin 0.5 mg/mL; [e]For 1, forskolin 1 mg/mL;[f]For 2, forskolin 2 mg/mL.

*$p<0.05$ when compared with the negative control group; †$p<0.05$ when compared with the EMS control group.

Results

A statistically significant increase in the DNA damage was observed in the larvae treated with the 1 mM ethyl methanesulphonate in comparison to the negative control group. Forskolin did not show genotoxic effect at lower concentrations, while at a concentrations of 1 and 2 mg/mL induced moderate increases in the total comet score when compared with the negative control. Further *in vivo* studies with other model organisms are needed before definitive conclusions about the absence of genotoxic potential of forskolin.

References

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research 175, 184-191.

Mukhopadhyay, I., Chowdhuri, D.K., Bajpayee, M., Dhawan, A., 2004. Evaluation of *in vivo* genotoxicity of cypermethrin *in Drosophila melanogaster* using the alkaline Comet assay. Mutagenesis 19, 85-90.

Acknowledgements

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreement No. 451-03-9/2021-14/200378 and Agreement No. 451-03-9/2021-14/200122).