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CYTOGENETIC EFFECTS OF CLOFIBRATE AND ITS CALCIUM SALT DERIVATIVE IN SWISS MALE MICE

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DRUGS are one of the largest groups of chemicals to which man is exposed and there is an ever-growing need to evaluate them for mutagenic activity. In the present study, clofibrate (ethyl *p*-chlorophenoxyisobutyrate), an anticholesterol drug, and its calcium derivative were studied for their mutagenic effect using spermatocyte meiotic chromosome analysis as there is very little information available regarding this aspect. There are a few reports that clofibrate and its derivatives which are potent peroxisome proliferators are carcinogenic[1]. In the present study the meiotic chromosomes were scored from each group of animals for each type of aberrations at 7, 15 and 30 days of treatment to examine the effect on early and late spermatogenic cycle.

Swiss male mice (5–6 weeks old) obtained from Biological Evans, Hyderabad were divided into groups containing 6 animals each and were orally administered with clofibrate 100, 200 and 400 mg/kg body wt for the time indicated. Animals were sacrificed 24 hr after the scheduled treatment. Control animals were given 4% gum acacia, in which the drug suspension was made. The metaphase chromosomes of spermatocytes of the testicular cells were prepared according to literature method[2]. Metaphases (500–600 per group) were screened for numerical (euploids and hyperploidy and hypoploidy) and structural (autosomal and xy univalencies and translocations) aberrations as described earlier [3–5]. Statistical analysis was carried out using X^2 test[6].

The analysis of spermatocyte I chromosomes at diakinesis of 1st meiotic metaphase showed direct morphological evidence of genetic alteration in the cells. Results obtained are presented in tables 1 and 2. At 7 days the incidence of total aberrations with clofibrate was 19.97%, 25.57% and 27.78% with 100, 200 and 400 mg/body wt respectively as compared with 11.62% in controls. However, the calcium salt of chlorophenoxyisobutyrate derivative gave rise to 17.71%, 19.59% and 25.48% with the above corresponding doses. Individual types of anomalies such as euploids, aneuploids and univalents were significant but the translocations were not significant at any dose.

As the duration of the treatment increased from 7 to 15 days the percentage of aberrations also increased and they were 26.64, 31.94 and 33.83 with clofibrate and 19.43, 20.93 and 23.93 with its calcium derivative at 100, 200 and 400 mg/body wt dosage as compared with 13.22% in controls. All the above values were significantly higher than the controls. As observed in 7-day treatment, the translocations were not significant but other individual abnormalities were significant. Only one dose of 100 mg of clofibrate and its calcium derivative were administered for 30 days as the other two higher doses were found to be lethal or toxic (the animals were sluggish and unhealthy looking) when administered for longer periods. Further treatment of 100 mg/body wt of the test compounds for 30 days gave rise to 28.32% and 23.95% of total aberrations with clofibrate and its calcium salt respectively. The aberration frequency was significant as compared to control frequency of 13.11%. Except translocations all other individual types of aberrations were highly significant than controls.

The above data clearly suggest that the increase in the dosage and duration of the treatment enhanced the rate of aberrations. It is also clear that the parent drug clofibrate is more potent in causing chromosomal aberrations than its new calcium derivative.

Table 1 *Frequency of chromosomal aberrations in spermatocytes of clofibrate administered and control mice*

Duration of treatment	Dose mg/kg body wt	Total meta-phases	Normal II		Abnormal II		Euploidy		Aneuploidy		Univalency		Translocations	
			No	%	No	%	No	%	No	%	No	%	No	%
7 days	Con	542	479	88.3	63	11.6	22	4.0	18	3.3	23	4.2	0	0
	100	581	465	80.0	116	19.9	37	6.3	31	5.3	48	8.2	0	0
	200	485	361	74.4	124	25.5	31	6.3	41	8.4	52	10.7	0	0
	400	540	390	72.9	150	27.7	42	7.7	39	7.2	68	12.7	1	0.2
15 days	Con	552	479	86.7	73	13.2	23	4.1	21	3.8	29	5.2	0	0
	100	533	391	73.3	142	26.6	38	7.1	43	8.0	59	11.0	2	0.4
	200	551	375	68.0	176	31.9	48	8.7	51	9.2	76	13.7	1	0.2
	400	522	348	66.6	174	33.3	43	8.2	57	10.9	74	14.1	2	0.4
30 days	Con	580	504	86.8	76	13.1	28	4.8	15	2.5	33	5.6	0	0
	100	519	372	71.6	147	28.3	36	6.9	41	8.0	69	13.2	1	0.2

Note: 1. Aneuploidy included hypoploidy (16II, 17II, 18II and 19II) and hyperploidy (21II, 22II and 23II)
2. Euploidy included 40II, 60II, 80II and 100II.
3. Univalencies included autosomal and sex (X, Y) chromosomes.
4. Translocations included rings and chains.
5. The percentage of each anomaly was determined: $\frac{\text{No. of each type of anomaly}}{\text{Total No. of metaphases scoped}} \times \frac{100}{1}$

Table 2 *Frequency of chromosomal aberrations in spermatocytes of clofibrate (Ca-Salt) administered and control mice*

Duration of treatment	Dose mg/kg body wt.	Total meta-phases	Normal II		Abnormal II		Euploidy		Aneuploidy		Univalency		Translocations	
			No	%	No	%	No	%	No	%	No	%	No	%
7 days	0	542	479	88.3	63	11.6	22	4.0	18	3.3	23	4.2	0	0
	100	525	432	82.2	93	17.7	31	5.9	25	4.7	37	7.0	0	0
	200	531	427	80.4	104	19.5	32	7.4	28	5.2	43	8.1	1	0.2
	400	518	386	74.5	132	25.4	39	7.5	41	7.9	52	10.0	2	0.4
15 days	0	552	479	86.7	73	13.2	23	4.1	21	3.8	29	5.2	0	0
	100	458	369	80.5	99	19.4	30	6.3	24	5.2	35	7.6	0	0
	200	482	381	79.0	101	20.9	31	6.4	29	6.0	41	8.5	1	0.2
	400	520	396	76.1	124	23.8	38	7.3	32	6.1	53	10.1	1	0.2
30 days	0	580	504	86.8	76	13.1	28	4.8	15	2.5	33	5.6	0	0
	100	522	397	76.0	125	23.9	39	7.4	32	6.1	54	10.3	1	0.2

Note: 1. Aneuploidy included hypoploidy (16II, 17II, 18II and 19II) and hyperploidy (21II, 22II and 23II)
2. Euploidy included 40II, 60II, 80II and 100II.
3. Univalencies included autosomal and sex (X, Y) chromosomes.
4. Translocations included rings and chains.
5. The percentage of each anomaly was determined: $\frac{\text{No. of each type of anomaly}}{\text{Total No. of metaphases scoped}} \times \frac{100}{1}$

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