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Cytogenetic Effects of Liv.52 on the Male Germline Cells of *Poekilocerus Pictus* (*Acrididae: Orthoptera)*

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ABSTRACT

Short-term cytogenetic effects of Liv.52 on the male germline cells of a pyrgomorphine grasshopper, Poekilocerus pictus were studied. Males received 4 µl, 8 µl and 12 µl of the drug through single injections. The effects of drug were studied at four different post-treatment periods i.e. 12h, 24h, 36h and 48 hours. Chiasma formation and various meiotic and spermatid abnormalities were the cytogenetic parameters employed in assessing the effects of Liv.52. Resultant data showed that the drug has no effect on genetic recombination as evaluated by chiasma frequency. While significant changes in individual meiotic and spermatid anomalies were not discernible, frequencies of total meiotic and spermatid anomalies were not discernible, frequencies of total meiotic and total spermatid abnormalities declined significantly with increasing dose of drug administration especially at longer post-treatment intervals. In a stimulating way, lowered incidence of diploid and polyploid spermatids are believed to be mainly, if not exclusively, responsible for declining frequencies of total spermatid abnormalities. Based on the available quantitative data, laggards and polyploidy are implicated in lowering the values of total meiotic and spermatid abnormalities. In the light of these findings, it is opined that Liv.52 functions as an antimutagenic agent by preserving the structural and functional integrity of the spindle and lowering the frequency of numerical mutations of chromosomes.

INTRODUCTION

Liv.52, a product of The Himalaya Drug Company, is an Ayurvedic drug with a herbal base. The formulation of the drug in the syrup form is as follows:

Each 2.5 ml of syrup contains extracts of:

Capparis spinosa	17 mg
Cichorium intybus	17 mg
Solanum nigrum	8 mg
Cassia occidentalis	4 mg
Terminalia arjuna	8 mg
Achillea millefolium	4 mg
Tamarix gallica	4 mg

Processed in the extracts of eleven other medicinal plants. It is a drug of choice for treating different hepatic disorders and is also favorably used in the management of anorexia of various etiology (Sheth *et al.*, 1963: Indira Bai *et al.,* 1971; Saxena, 1971). Apart from the extensive clinical and biochemical studies made, histological investigations have been carried out on the liver to study the impact of the drug on the regeneration of hepatic parenchyma (Gupta *et al.,* 1972; Patney *et al.,* 1976; Prasad, 1976; Kishore *et al.,* 1978). The drug's efficacy in radioprotection is also reported by Saini *et al.,* (1984 and 1985). In spite of these extensive studies on somatic cells, no attempt has been made to study its effect on the germline cells. Therefore, the present work was undertaken to

analyse the short-term effects of the drug on the male germline cells of a grasshopper, *Poekilocerus pictus*, with an emphasis on cytogenetic parameters.

MATERIALS AND METHODS

Adult male individuals of the short horned pyrogomorphine grasshopper, *Poekilocerus pictus* (Acrididae: Orthoptera), which has a diploid chromosome complement, 2n=19, constituted the experimental material. Individuals weighing about 3.0 gms were administered with various doses of Liv.52 syrup. The LD_{50} value for *P. pictus* was found to be 15 µl for Liv.52 syrup. Hence, doses of 4 µl, 8 µl and 12 µl were administered to these grasshoppers in single injections. Four groups each, containing 20 individuals were used for experiments. Of these, three groups received 4 µl, 8 µl and 12 µl of the syrup and the remaining group served as control. After the administration of the drug, five individuals from each group were sacrificed at regular intervals of 12 h, 24 h, 36 h and 48 hours. The testes were removed after vivisection, fixed in acetic acid-methanol (3:1) and then preserved in 70% alcohol. Squash preparations of the testes were made after staining them with Heidainhain'' Iron Haematoxylin.

The cytogenetic survey was carried out by analysing the chiasma frequency, various meiotic and spermatid abnormalities in the control and treated individuals. The data were subjected to appropriate analysis of variance and the results were tabulated separately.

OBSERVATIONS

Chiasma frequencies calculated for different treated groups and controls at various post-treatment periods are given in Table 1. The results indicate no appreciable difference, between different groups of individuals and the control at varied durations after treatment. Data in Table 2 further confirm these observations.

Table 1: Chiasma frequency in diplotene cells of Liv.52 treated *Poekilocerus pictus*

Time in hours	Diplotene cells scored	Mean chiasmata			
		Control	4 µl	8 µl	12 µl
12	50	16.94 ± 0.09	17.10 ± 0.20	17.02 ± 0.16	17.00 ± 0.20
24	50	17.14 ± 0.20	17.00 ± 0.16	17.08 ± 0.19	17.12 ± 0.15
36	50	16.80 ± 0.31	17.00 ± 0.18	17.14 ± 0.25	17.04 ± 0.25
48	50	17.06 ± 0.24	17.10 ± 0.21	17.10 ± 0.17	16.98 ± 0.19

Univalent formation, anaphase laggards, bridges, polyploidy in metaphase II and anaphase II were some of the meiotic anomalies encountered. The incidences of these anomalies are given in Table 3. All anomalies except bridges contribute to numerical variations whereas bridges arise from the breakage and reunion of chromosomes.

Table 2: Analysis of variance of chiasma frequency in Liv.52 treated *Poekilocerus pictus*

SV	DF	SS	MSS	F ratio
Dose	3	0.11	0.04	0.17
Duration	3	0.12	0.04	0.18
Interaction	9	0.34	0.04	0.18
Error	64	13.53	0.21	

Figures in Table 3 reveal that the individual and total meiotic anomalies at 12 hours after treatment are not significantly different from control. In the remaining post-treatment periods, anaphase laggards and second meiotic polyploidy show a declining trend with increasing time lag as well as at increasing concentration of Liv.52. Frequency of total meiotic anomalies for 8 µl treatment decreases from 0.90% at 12 hours to 0.70% at 36 and 48 hours. Similarly, the incidence declines from 0.90% at 12 hours to 0.70% at 24 hours and to 0.50% at 36 and 48 hours for 12 µl treatment.

Analysis of these data, however, shows that the above indicated declining trend at longer post-treatment time intervals is not statistically significant (Table 4) either for anaphase laggards and second meiotic polyploidy or for total meiotic anomalies. When the total meiotic anomalies are compared between control and different treated groups, it is seen that there is a progressive and significant (p<0.05) decrease with an increase in dose of treatment of the drug from 24 hours after treatment.

Table 3: Frequency (%) of male meiotic anomalies in Liv.52 treated *Poekiloerus pictus*

Time in hours	Meiotic stages	Cells scored	Anomalies	Doses			
				Control	4 µl	8 µl	12 µl
12	Metaphase I	500	Univalent	0.4	0.6	0.4	0.2
	Anaphase I	500	Bridges	0.4	0.2	0.4	0.4
			Laggards	0.6	0.6	0.6	0.4
	Telophase I	500	Laggards	0.6	0.6	0.6	0.8
	Metaphase II	500	Polyploidy	0.6	0.8	0.6	0.6
	Anaphase II	500	Bridges	0.6	0.6	0.4	0.4
			Laggards	0.6	0.8	0.6	0.6
			Polyploidy	0.6	0.6	0.6	0.4
	Telophase II	500	Laggards	0.6	0.6	0.8	0.8
		3000	Total meiotic anomalies	0.80	0.90	0.90	0.90
24	Metaphase I	500	Univalent	0.4	0.6	0.8	0.4
	Anaphase I	500	Bridges	0.4	0.4	0.2	0.4
			Laggards	0.8	0.8	0.6	0.6
	Telophase I	500	Laggards	0.6	0.6	0.8	0.6
	Metaphase II	500	Polyploidy	1.0	0.8	0.8	0.6
	Anaphase II	500	Bridges	0.6	0.8	0.6	0.4
			Laggards	0.8	0.8	0.6	0.6
			Polyploidy	0.6	0.6	0.4	0.2
	Telophase II	500	Laggards	0.6	0.8	0.8	1.0
		3000	Total meiotic anomalies	0.9	1.0	0.90	0.70
36	Metaphase I	500	Univalent	0.4	0.6	0.4	0.2
	Anaphase I	500	Bridges	0.4	0.4	0.2	0.2
			Laggards	0.8	0.8	0.6	0.2
	Telophase I	500	Laggards	0.6	0.4	0.6	0.6
	Metaphase II	500	Polyploidy	0.8	0.6	0.6	0.4
	Anaphase II	500	Bridges	0.6	0.6	0.4	0.4
			Laggards	0.8	0.6	0.4	0.2
			Polyploidy	0.6	0.6	0.6	0.4
	Telophase II	500	Laggards	0.6	0.6	0.4	0.4
		3000	Total meiotic anomalies	0.90	0.80	0.70	0.50
48	Metaphase I	500	Univalent	0.4	0.4	0.6	0.2
	Anaphase I	500	Bridges	0.4	0.4	0.4	0.2
			Laggards	0.6	0.6	0.4	0.4
	Telophase I	500	Laggards	0.4	0.4	0.6	0.2
	Metaphase II	500	Polyploidy	0.8	0.6	0.8	0.6
	Anaphase II	500	Bridges	0.6	0.4	0.4	0.4
			Laggards	0.8	0.6	0.4	0.4
			Polyploidy	0.8	0.6	0.4	0.4
	Telophase II	500	Laggards	0.6	0.6	0.4	0.2
		3000	Total meiotic anomalies	0.80	0.70	0.70	0.50

Data found in table 5 indicate that among the three types of spermatid abnormalities scored, diploid and polyploid spermatids tend to decrease with increase dose of treatments. This is true for all the durations of treatments. The incidence of total spermatid abnormalities is significantly lower in treated animals than in controls. Statistical comparisons of data (Table 6) with reference to individual spermatid abnormalities show that there is no significant variation between control and treated groups and also between individuals that received different doses of Liv.52. In contrast, the incidence of total spermatid abnormalities showed a significant (p<0.005) dose related decrease. The higher the dose of Liv.52 treatment, lower the frequency of total spermatid abnormalities. Thus, there exists a close correlation between the pattern of incidence of total meiotic and total spermatid abnormalities.

Table 4: F ratios for the comparison of effects between doses, between time intervals and between dose and time interactions with reference to male meiotic anomalies in Liv.52 treated *Poekilocerus pictus*

Anomalies scored	F ratios		
	Dose df (3,64)	Time df (3,64)	Dose × Time Interaction df (9,64)
M – I Univalent	0.75	0.28	0.14
A – I Bridges	0.17	0.05	0.21
A – I Laggards	0.77	0.53	0.13
T – I Laggards	0.10	0.77	0.13
M – II Polyploidy	0.62	0.45	0.14
A – II Bridges	0.72	0.27	0.12
A – II Laggards	1.18	0.59	0.20
A – II Polyploidy	1.22	0.17	0.14
T – II Laggards	0.10	1.29	0.28
Total meiotic anomalies	3.32*	1.24	0.36

*p<0.05

Table 5: Frequency (%) of spermatid abnormalities in Liv.52 treated *Poekilocerus pictus*

Time in hours	Spermatids scored	Spermatid abnormalities scored	Dose			
			Control	4 µl	8 µl	12 µl
12	2000	Diploid and polyploid spermatids	5.85	5.60	5.10	4.75
		Microspermatids	0.15	0.25	0.25	0.10
		Abnormal shaped spermatids	0.65	0.70	0.65	0.55
		Total spermatid abnormalities	6.65	6.55	6.00	5.40
24	2000	Diploid and polyploid spermatids	6.06	5.10	5.00	4.70
		Microspermatids	0.20	0.25	0.20	0.10
		Abnormal shaped spermatids	0.75	0.60	0.65	0.50
		Total spermatid abnormalities	7.00	5.95	5.85	0.53
36	2000	Diploid and polyploid spermatids	5.95	5.35	4.95	4.85
		Microspermatids	0.20	0.20	0.15	0.10
		Abnormal shaped spermatids	0.65	0.80	0.65	0.60
		Total spermatid abnormalities	6.80	6.35	5.75	5.55
48	2000	Diploid and polyploid spermatids	5.65	5.15	5.10	4.90
		Microspermatids	0.25	0.25	0.20	0.15
		Abnormal shaped spermatids	0.75	0.90	0.85	0.70
		Total spermatid abnormalities	6.65	6.30	6.15	5.75

DISCUSSION

Estimates of chiasma frequencies in Liv.52 treated individuals indicate that no variation in genetic recombination has been brought about by the drug. It is a known fact that chiasma formation involves chromosome breakage and X-type of reunion and also that the localization and frequency of chiasma formation are regulated both by genetic and environmental components (Gale and Rees, 1970; Rahiman and Rajasekarasetty, 1971; Shaw, 1971). That Liv.52 has neither increased nor decreased the genetic recombination in any manner, therefore, indicates that it

does not alter the genetic milieu or modify the internal environment so as to change the expression of polygenes, which regulate chiasma formation.

Previous studies in acridids have established that a low incidence of meiotic anomalies spontaneously occur in the male germline cells of several acridids, including *P. pictus* (Sharma *et al.,* 1962; John and Lewis, 1965; Rahiman, 1971; Gururuj 1974). This shows that the replicative cycle of chromosomes as well as their behavior during cell division do not maintain absolute fidelity but are error prone. These errors no doubt, in the long run are beneficial to the species concerned, since they introduce genetic variations on which natural selection operates to mould new adaptive genotypes. The exposure of meiotic cells *in vivo* to an extraneous agent like Liv.52 may influence the incidence of these errors. If the effects were to be adverse, a correlated higher quantum of meiotic anomalies will result. This is detrimental to the organism, because several of these anomalies, which are nonadaptive, are liable to reduce its reproductive potential. Conversely, if the occurrence of chromosome variations is reduced in the presence of Liv.52, then the drug can be considered as an antimutagenic agent.

Table 6: F ratios for the comparison of effects between doses, between time intervals and between dose and time interactions with reference to spermatid abnormalities in Liv.52 treated *Poekilocerus pictus*

Spermatid abnormalities	F ratios		
	Dose df (3,64)	Time df (3,64)	Dose × Time Interaction df (9, 64)
Diploid and polyploid spermatids	0.42	0.03	0.12
Microspermatids	1.65	0.29	0.17
Abnormal shaped spermatids	0.56	1.92	0.07
Total spermatid abnormalities	3.43*	1.04	0.38

*$p<0.05$

Data on various meiotic anomalies scored in Liv.52 treated individuals show that anaphase laggards and second meiotic polyploidy consistently have a low incidence, especially at longer post-treatment intervals and also at higher doses of drug treatment. A similar but highly pronounced trend was noticed with reference to the total meiotic anomalies also. These observations are further supplemented by the data obtained on spermatid abnormalities wherein the incidence of total spermatid abnormalities was significantly reduced in treated individuals. The combined data of meiotic and spermatid anomalies point that significant reduction in total anomalies is achieved by a corresponding reduction in numerical variations. Many of the numerical variations arise due to disturbances in the structure and function of the spindle (Henderson, 1962; Gururaj and Rajasekarasetty, 1969 and 1976). Since the incidence of numerical variations has declined, it can be inferred that Liv.52 fortifies spindle structure and function in such a way that laggards become less numerous and spindle breakdown and its consequent ploidy are prevented. Thus, Liv.52 can be construed as an antimutagenic agent, which effectively reduces chromosomal variations by way of suppression of numerical mutations of chromosomes. It is also apparent that lack of reduction in the frequencies of bridges and chiasmata which involve chromosome breakages underlie the fact that Liv.52 does not function as an anticlastogenic agent or enhance the efficacy of genetic repair mechanisms in short term treatment modes.

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