Effects of Delta-9-Tetrahydrocannabinol (Marijuana) and Diacetyl Morphine (Heroin) on Human Chromosomes

Dolores A. Ramirez
Professor
University of the Philippines Los Baños
College, Laguna

M.C.R. Nierras and
S.M. Madrid-Scheller
Former Biology Students
University of the Philippines Los Baños
College, Laguna

ABSTRACT

The effects on human chromosomes of delta-9-tetrahydrocannabinol extracted in methanol from marijuana leaves and diacetylmorphine or heroin were studied using leukocyte cultures obtained from ten male and ten female volunteers.

The number of chromosomal aberrations in the treated samples showed statistically significant deviation from the control groups. Increased frequencies of aberrant chromosomes were observed as drug concentration was increased, indicating dose dependency of drug action. At all concentrations, the larger chromosomes showed higher frequencies of aberrations than the small ones. The chromosome aberrations include chromatid and isochromatid breaks and gaps, loose pairing of sister chromatids, and terminal and intercalary deletions resulting in chromosome fragmentation. Irregular condensation patterns of chromosomes were also observed. The types of chromosome aberrations were independent of drug concentration.

INTRODUCTION

In recent years, there has been increased concern regarding the widespread abuse of various psychoactive drug preparations, especially by young people. The problems and disabilities associated with the use of drugs are numerous. This study was focused on the effect of the two more popular psychoactive drugs, marijuana and heroin, on human chromosomes.

Marijuana is the crude preparation of flowering tops, leaves, seeds and stems of the Indian hemp, *Cannabis sativa* L.. It contains natural cannabinoids which may be interconverted by a number of procedures including combustion. The major psychoactive component of marijuana is delta-9-tetrahydrocannabinol (Δ-9-THC) (Gaoni and Mechoulam, 1974; Mechoulam, 1970).

On the other hand, diacetyl morphine or heroin is a morphine analogue. It is more potent than morphine due to drug latentiation: the blocking of hydroxyl groups by biologically removable lipophylic groups thereby facilitating rapid entry into the central nervous system (Smith and Cole, 1976).

This study was undertaken to determine the effects of marijuana and heroin on *in vitro* cultures of human leucocytes.

MATERIALS AND METHODS

Dried marijuana leaves and heroin of 92.5% purity were obtained from stocks of PC Crime Laboratory at Camp Crame, Quezon City. From the marijuana samples, Δ-9-THC was extracted using the procedures of Schultz and Haffner (1960). For each drug, the treatments consisted of three concentrations and the control which received sterile saline solution only. The Δ-9-THC treatments were 0, 50, 100 and 250 ng/ml while that of heroin were 0, 0.025%, 0.05%, and 0.075%.

Peripheral blood leukocytes were obtained from volunteers, five males and five females of ages ranging from 18 to 20 years old for each of the drugs. Three to four drops of blood were obtained from each volunteer which were extruded directly into the previously prepared culture media (Gibco Cat. Nos. 167 D and 167 L). The blood cultures were incubated at 37° C for 48 hours before introducing the different treatments. After treatment, the blood cultures were then harvested; cells were fixed and prepared for cytological analyses. All the slide preparations were examined and only the cells with a minimum of 45 centromeres were considered. Fifty to 100 well-spread metaphase cells per dosage per individual were examined. The

total number of cells showing chromosome aberrations were counted and examined for identification of the types of aberration.

RESULTS

Delta-9-Tetrahydrocannabinol (Marijuana)

Results obtained from ten individuals (five males and five females) of ages 19 to 20, showed a significant increase in the number of aberrant cells with increasing concentrations of the drug (Table 1). Cells showing two or more chromosome aberrations were classified as multiple-chromosome damaged cells while those showing one aberrant chromosome were classed as single chromosome damaged cells. Note the higher frequency of single chromosome aberrations at 50 ng/ml only; at higher doses, multiple chromosome aberrations predominated. The predominance of multiple chromosome aberrations is due to increased frequencies of loose sister chromatid pairing and irregular chromosome condensation.

Table 1. Average number of cells with single and multiple chromosome aberrations at different Δ-9-THC concentrations. (Based on 50 metaphase cells from each of 5 male and 5 female volunteers).

GROUP	CONCENTRATION OF A-9-THC (ng/ml)							
	0		50		100		250	
	Single	Multiple	Single	Multiple	Single	Multiple	Single	Multiple
Males	3.4	1.0	8.0	4.0	12.0	19.8	12.6	27.8
Females	3.2	0.6	7.4	4.8	12.6	17.6	13.4	29.2
Mean	3.3	0.8	7.7	4.4	12.3	18.7	13.0	28.5

Table 2 shows that the larger chromosomes groups were easily affected by the Δ-9-THC concentrations than the smaller ones, with Group A (Chromosomes 1, 2 and 3) being most affected. Futhermore, increasing concentration of the drug effected increasing frequencies of aberrations for all groups.

Table 2. Average number of chromosome aberrations (all types) in human leukocytes at different Δ-9-THC concentrations. Based on 50 metaphase cells from each of five male and five female volunteers).

Δ-9-THC CONCENTRATION (ng/ml)	CHROMOSOME GROUP							MEAN
	A	B	C	D	E	F	G	
0	2.8	2.8	2.8	2.5	2.2	2.8	2.2	2.56
50	9.6	8.2	8.2	0.6	8.6	7.2	6.4	6.97
100	33.8	36.5	36.5	32.4	31.1	32.4	31.1	33.40
250	53.4	48.4	46.6	35.0	33.4	35.0	35.0	40.97

Table 3 shows the types of aberrations observed. The data indicate that the types of aberrations are independent of Δ-9-THC concentration. This means that for any given concentration, there are equal chances of inducing either a chromatid type or a chromosome type lesion. The increasing drug concentration also increased chromatin condensation with the 250 ng/ml treatment showing extensive condensation (Table 4).

Table 3. Type and number of chromosome aberrations in human leukocytes caused by D-9-THC. (Based on 50 metaphase cells from each of five male and five female volunteers).

Δ-9-THC CONCENTRATION (ng/ml)	TYPE OF ABERRATION						
	Chromatid Type			Chromosome Type			
	Single gap	Single break	Total	Iso-gap	Iso-break	Loose pairing	Total
0	5.0	3.0	8.0	2.5	1.5	4.5	8.5
50	6.4	3.6	10.0	1.8	3.6	8.2	13.6
100	5.4	13.5	18.9	5.4	0	32.4	37.8
250	26.7	6.7	33.4	3.0	0	46.7	49.7

Figs. 1 and 2 show normal male and female cells while Figs. 3, 4 and 5 show the different chromosomal aberrations caused by Δ-9-THC: chromatid gaps or intercalary deletions, loose sister-chromatid pairing and irregular chromatin condensation.

Table 4. Average number of cells showing irregular condensation pattern at different Δ-9-THC concentrations (Based on 50 metaphase cells from each of five male and five female volunteers).

GROUP	Δ-9-THC CONCENTRATION (ng/ml)			
	0	50	100	250
Males	2.0	6.4	25.6	27.8
Females	2.6	6.4	23.0	29.0
Mean*	2.9 a	8.4 a	24.9 b	28.4 b

*Means followed by the same letter are not significantly different at 1% level.

Fig. 1. Morphology of a metaphase cell of normal male chromosomes showing 2n = 46.

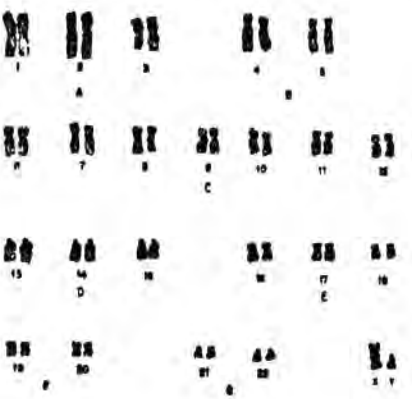

Fig. 1a. Karyotype of a metaphase cell of a normal male showing 2n = 46.

Fig. 2. Morphology of a metaphase cell of a normal female chromosomes showing 2n = 46.

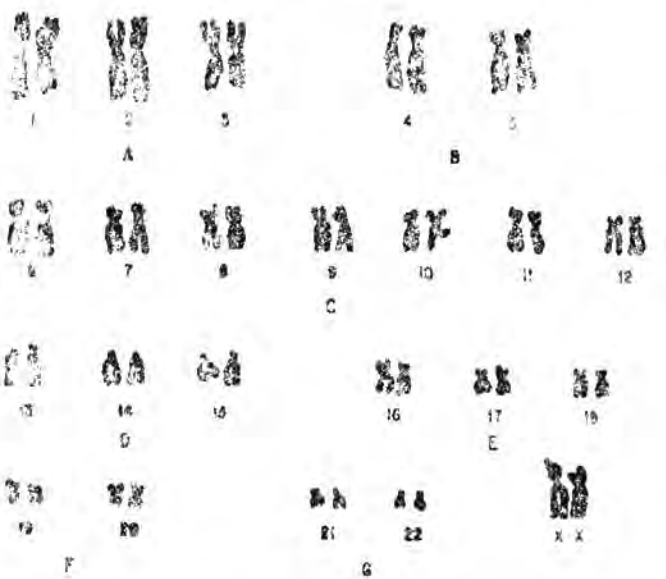

Fig. 2a. Karyotype of metaphase cell of a normal female showing 2n = 46.

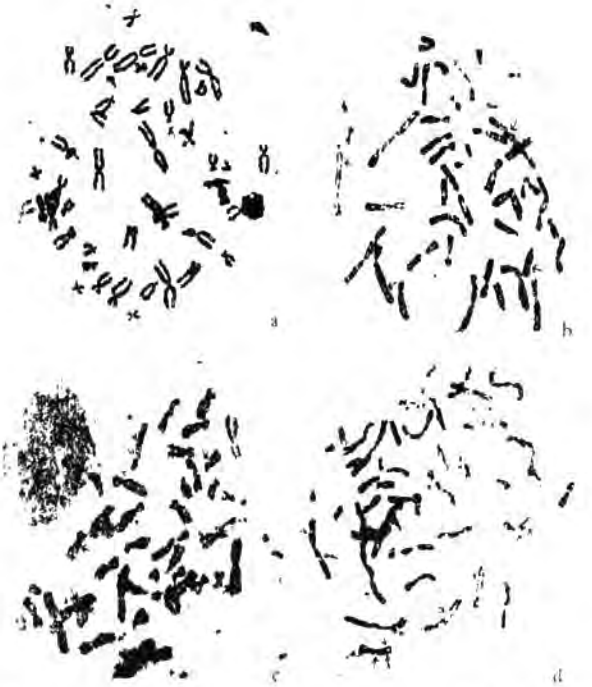

Fig. 3. Chromatid aberrations of leukocytes treated with Δ-9-THC: (a) control (b) 50 ng/ml (c) 100 ng/ml. (Arrows point to chromatid gaps).

Fig. 4. Loose pairing of chromosomes in treated cultures: (a) 50 ng/ml and (b) 100 ng/ml. (Arrows point to loosely paired chromosomes).

Fig. 5. Irregular chromatid condensation patterns of leukocytes treated with Δ-9-THC: (a) control, (b) 50 ng/ml, (c) 100 ng/m, and (d) 250 ng/ml.

Analysis of data showed that the sex was independent of drug condensation, i.e. both males and females showed about the same responses to treatment (Table 5). Court Brown (1967) found that the effect of sex on the frequency of chromosomal aberration is closely related to hormonal changes associated with age. The age range of the volunteers is extremely limited, 19 to 20 years old only. Therefore it is not possible to detect the effect of sex nor age in this experiment.

Table 5. Effect of different Δ-9-THC concentrations and sex on the number of aberrant cells in human leukocytes. (Based on 50 metaphase cells from each of five male and five female volunteers).

SEX	CONCENTRATION (ng/ml)				MEAN[a]
	0	50	100	250	
Female	3.8	12.2	30.2	42.6	22.15a
Male	4.4	12.0	31.8	10.4	22.2 a
Mean[b]	4.1 a	12.1 b	31.0 c	41.5 d	

[a]Means for comparing the effect of sex

[b]Means for comparing the effect of marijuana concentrations

Note: Means followed by the same letter are not significantly different at 1% level.

Diacetyl Morphine (Heroin)

Results showed that heroin caused a significant increase of chromosomal aberrations with increasing concentration of heroin (Table 6). The larger chromosomes, Groups A, B and C had higher frequencies of aberrations compared to that of Groups D, E and F. The chromatid type of aberrations consisting of single chromatid gaps and breaks (Table 7 and Fig. 6) were less frequent than the chromosome types (Fig. 7). Among the chromosome-type aberrations, loose pairing of sister chromatids were predominant. Comparing the number of cells exhibiting chromosome-type aberrations with those of chromatid-type aberrations, no significant differences were observed at 0.025% and 0.05% heroin dosage. However, at 0.075% heroin concentration, cells with chromatid-type aberration occurred in greater frequencies. With increasing heroin concentrations, it was observed that certain localized segments of chromosomes became the regions preferentially damaged by heroin. This caused a marked decrease in the number of chromosomes with loose sister chromatid pairing. It was also observed that with increased drug dosage, aberrations in the long arm, and the telomeres were more than that of short arm of the chromosomes.

Table 6. Average number of chromosome aberrations in human leukocytes at different heroin concentrations. (Based on 100 metaphase cells from each of five male and five female volunteers).

HEROIN CONCENTRATION (%)	CHROMOSOME GROUP							MEAN
	A	B	C	D	E	F	G	
Control	2.9	2.4	1.6	.2	-	-	-	1.01
0.025	24.8	17.2	11.5	1.0	-	0.1	-	7.80
0.050	25.8	15.3	13.2	1.7	0.5	0.1	-	8.09
0.075	29.6	25.7	20.6	3.0	0.5	0.1	-	11.36

Table 7. Type and number of chromosome aberrations in human leukocytes caused by heroin. (Based on 100 metaphase cells from each of five male and five female volunteers).

HEROIN CONCEN-TRATION (%)	TYPE OF ABERRATION						
	Chromatid Type			Chromosome Type			
	Single gap	Single break	Total	Iso-gap	Iso-break	Loose pairing	Total
Control	2.4	1.8	4.2	-	-	3.6	3.6
0.025	8.3	6.8	15.1	3.0	0.1	27.9	31.0
0.50	11.8	11.7	23.5	6.9	2.1	23.0	32.0
0.075	24.4	20.4	44.8	7.6	4.1	21.3	33.0

It was also observed that the mitotic anomalies due to chromosome breakages were accompanied by loss of chromosome fragments. At 0.05% and 0.75% heroin concentrations, aberrations such as terminal deletions, intercalary deletions and acentric rings were observed. These are loosely grouped together as acentric fragments (Fig. 8). Chromosome fragmenttaion and degeneration were observed highest at 0.075% heroin concentration.

As observed in Δ-9-THC treated cells, there were no association between drug concentration and sex as well as age. This too may be due to the fact that the limited age range of the volunteers (18 to 20 years old) could not discriminate their effects.

Fig. 6. Chromosome and chromatid-type of aberrations observed in leukocyte cultures caused by exposure to 0.075% heroin of (a) female and (b) male treated subjects. Heavy arrows point to chromosome-type aberrations: (a) isochromatid break, and (b) isochromatid gap. Light arrows indicate chromatid-type aberrations (a) single and (b) single gap.

Fig. 7. Leukocyte cell from a blood culture of a treated female showing multiple chromosome aberrations: single gaps and isochromatid gaps involving chromosome groups A and C. (Indicated by arrows).

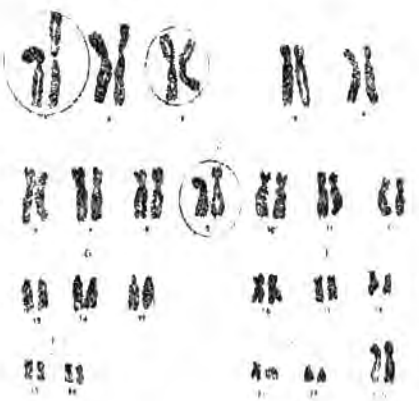

Fig. 7a. Karyotype of metaphase cell of treated female leukocyte showing aberrations involving chromosome groups A and C.

Fig. 8. Leukocyte cultures treated with 0.075% heroin concentration showing metaphase chromosome fragmentation and degeneration as shown by arrows.

DISCUSSION

Increasing concentration of both Δ-9-THC and heroin effected increased frequencies of aberrations of all groups of the human chromosomes because more drugs become available to interact with chromatin materials. The same trend was observed by Villegas and Ramirez (1979) among Diazepam (Valium) treated leukocyte cultures. The apparent non-random distribution of aberrations in chromosome groups was observed, with Group A chromosome showing the highest percentage of damage. By virtue of their sizes and density distributions, the larger chromosomes are more readily reactive to Δ-9-THC or heroin than the smaller groups.

The independence of the type of chromosome aberrations from the concentration of Δ-9-THC or heroin implies that these drugs affect DNA at any time during the cell cycle. The chromosome-type aberrations are results of damages to the DNA prior to replication and the chromatid-type aberrations are due to damages during and after DNA synthesis (Brinkley and Hittelman, 1975).

The frequencies and types of chromosomal aberrations and fragmentation caused by Δ-9-THC and heroin argue against the survival of aberrant cells. On the other hand, there are enough evidence that cancers are associated with chromosomal aberrations and that enough mutant cells may tip the threshold for the development of malignancy. It is, therefore, possible that in addition to many disabilities associated with marijuana and heroin, these drugs of abuse may be acting as clastogen or chromosome breakers and therefore mutagens with carcinogenic properties.

REFERENCES

Brinkley, B.R. and W.N. Hittolman. 1975. Ultrastructure of mammalian chromosomal aberrations. Int'l. Rev. Cytology 42:49-98.

Court Brown, W.M. 1967. Human population cytogenetics 5:28-29.

Gaoni, Y. and R. Mechoulam. 1974. Isolation, structure and partial synthesis of an active constituent of hashish. J. Amer. Chem. Soc. 86:1646-1647.

Mechoulam. 1970. Marijuana chemistry. Science 168:1159-1161.

Schultz, O.E. and G. Haffner. 1960. Arch. Pharm. 293.

Smith, D.A. and W.J. Cole. 1976. Identification of an arylesterase as the enzyme hydrolyzing diacetyl morphine in human plasma. Jour. Pharmacy & Pharmacology *23*:843-844.

Villegas, A.N. and D.A. Ramirez. 1979. Effects of diazepam (Valium) on human chromosomes. Kalikasan, Philipp. J. Biol. 8:1-12.