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Original Article

Cytogenetic Evaluation of the Physiological Saline Extract of a Newly Developed Dental Material "ORMO-48"

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ABSTRACT

The ORMO-48 is a new indigenous material for dental applications, developed by the Dental Products Laboratory of our Institute. The aim of the present study was to evaluate the genotoxic effect of an indigenously developed dental material in Swiss albino mice. The genotoxic effect was evaluated by micronucleus and chromosomal aberration tests. Two grams of dental material was extracted in 10.0 ml of physiological saline at 70 °C for 24 h. The extract was cooled to room temperature and was used for the experiment. The experimental designed had three groups each (six mice in each group) for micronucleus and chromosomal aberration tests. The first, second, and third groups were given a single exposure of physiological saline alone (control), dental material's extract (test), and cyclophosphamide (positive control) respectively for micronucleus and chromosomal aberration tests. The result of the study indicated that, the percentage of micronucleated PCE (polychromatic erythrocytes) and NCE (normochromatic erythrocytes) induced by the dental material (extract) treated group was well comparable with control group, whereas the positive control induced significantly high ($P < 0.001$) micronucleated PCE when compared to control. The PCE and NCE ratio of the dental material extract treated group was similar to that of control group. The chromosomal anomalies such as chromatid/chromosomal breaks, centric rings, exchanges, dicentric, and acentric fragments were evaluated. The result showed that the anomalies of the dental material extract treated group were similar to control group, however, significant anomalies were observed in the cyclophosphamide treated group. Hence, the present study concluded that the indigenously developed biocompatible dental material, ORMO-48 is non genotoxic at our laboratory conditions.

Key words: Chromosomal aberrations, dental material, genotoxicity, micronuclei

INTRODUCTION

Genotoxicity testing is generally used for hazard identification with respect to DNA damage[1,2] and the damages can be manifested in the form of gene mutation, structural chromosomal aberration, recombination, and numerical changes. These changes are responsible for heritable effects on germ cells and impose risks to future generations.[3] Chromosomal aberrations and micronuclei are useful biomarkers of DNA lesions and frequently used in genotoxicity testing. It is also suggested that chromosome aberrations are early predictors of cancer risk.[4] The micronucleus and chromosomal aberration tests were carried out to evaluate consistent and specific cytogenetic screening for drugs, chemicals, medical devices or implants used for human health care system. Micronuclei originate from chromatin which for different reasons lags in the anaphase stage of cell division. They are considerably smaller than the principal nucleus and are therefore called micronuclei. Lagging can be due to chromosome breakage

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or due to malfunctioning of the spindle apparatus. Micronuclei can occur in any cell type of proliferating tissues and are most easily recognized in cells lacking the main nucleus, namely erythrocytes. The frequency of micronuclei can be most easily evaluated in young erythrocytes shortly after the main nucleus is expelled. These young erythrocytes are termed as polychromatic erythrocytes (PCE) and are distinguished from the mature normochromatic (NCE) erythrocytes.[5,6] Chromosomal aberrations are carried out to evaluate the frequency of structural chromosomal aberrations like gaps, breaks and translocations. Chromosomal aberrations are due to lesions in DNA which lead to discontinuities of the DNA double helix, the evidence for double stranded breaks being the ultimate DNA lesion for chromosomal aberrations. Most of the aberrations are lethal to cells in which they occur either because of loss of vital genetic information or because, for mechanical reasons, they cannot survive mitosis. Ionizing radiation induces chromosome-type aberrations (symmetric aberrations) such as dicentrics, inversions, ring chromosomes in the G0 or G1 stage of the cell cycle. Where as chromatid type aberrations (asymmetric aberrations) such as breaks 4 and gaps are induced during the S or G2 stage (during or after replication) of cell cycle. Most of the chemical mutagens are S-dependent clastogens and induces chromatid-type aberrations.[7,8] In the present study an attempt was made to evaluate the cytogenetic effect of an indigenously developed dental composite material ORMO-48 in Swiss albino mice.

MATERIALS AND METHODS

Sterile disposable syringes, needles (23-25 gauge), physiological saline, cyclophosphamide, fetal calf serum, centrifuge tubes, may-grunwald stain, giemsa stain, hypotonic solution (KCl), methanol, and acetic acid.

Dental material

The ORMO-48 is a new indigenously developed material for dental application (filling), by the Dental Products Laboratory of SCTIMST. This dental restorative material is an organically modified ceramic composite paste.

Extraction of dental material

Two grams of ORMO-48 dental composite material was extracted in 10 ml of the physiological saline. The extraction was carried out at 70°C in an incubator shaker for 24 h.[9] Twenty four hours after the incubation, the extract was collected in a sterile beaker and was used for the micronucleus and chromosomal aberration studies.[10-12] Physiological saline processed in similar manner was used as control.

Experimental design

The design of the experiment is mentioned in the Table 1.

Animals

Swiss albino mice (SCTIMST), weighing 17-23g were used through out the experiment and were maintained on a standard laboratory feed and water *ad libitum*. They were housed in groups of six mice per cage. The room was maintained at temperature 20±3°C and humidity 40 - 60%. All the procedures were carried out in accordance with National regulations and Institutional Animal Ethics Committee guidelines. The experiment design had six groups (Group I to III for micronucleus and Group IV to VI for chromosomal aberration) and each group consisted of six Swiss albino mice [Table 2]. Group I and IV were control groups and received only physiological saline (50 ml/kg), Group II and V received physiological saline extract (50 ml/kg) of dental material (ORMO-48) and Group III and VI were given cyclophosphamide (20 mg/kg) as positive control by intraperitoneal administration. Twenty four hours after the administration, all the animals were sacrificed by cervical dislocation.

Micronucleus test

The animals (Group I, II, III) were sacrificed 24 h after the administration of control, test, and positive control samples. Immediately afterwards the bone marrow from both femurs of each mouse was collected by aspiration and suspended in fetal calf serum. Following centrifugation, bone marrow smears were prepared from the sediment cells in each case. The dried slides were fixed in methanol and stained with undiluted and diluted May- Grunwald stain and counter stained with Giemsa stain. Two thousand polychromatic

Table 1: Experimental design

Group	No. of animals	Dose	Samples	Route of injection	Bone marrow callection after (hrs)
			Micronuclei		
I	6	50 ml/kg	Physiological saline	Intraperitoneal	24
II	6	50 ml/kg	Dental material ORMO-48	Intraperitoneal	24
III	6	20 mg/kg	Cyclophosphamide	Intraperitoneal	24
			Chromosomal aberration		
IV	6	50 ml/kg	Physiological saline	Intraperitoneal	24
V	6	50 ml/kg	Dental material ORMO-48	Intraperitoneal	24
VI	6	20 mg/kg	Cyclophosphamide	Intraperitoneal	24

Group I: Control, II: Text, II Positive control (Micronucleil test), Group IV: Control, V: test, VI: Positive control

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erythrocytes (PCE) were analyzed microscopically in each preparation to assess the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was also recorded.[5]

Chromosomal aberration test

The animals (Group IV, V, VI) were sacrificed 24 h after the administration of control, test, and positive control samples. Immediately afterwards the bone marrow from both femurs of each mouse were collected by aspiration and suspended in physiological saline.

Following centrifugation, the cell button was treated with hypotonic solution (0.075 M KCl) for 15 min at 37 °C. After centrifugation, the cell button was fixed in methanol acetic acid (3:1) and smears were prepared. The dried slides were stained with Giemsa stain. One hundred well spread metaphases were analyzed microscopically (100×) in each preparation to evaluate the evidence of chromosomal anomalies.[13-15].

Statistical analysis

All the results were expressed as mean ± SE. The total variation and difference among means were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis. The *P* value less than 0.05 were considered significant.

RESULTS AND DISCUSSIONS

The stained slides were analyzed at 100× magnification using oil immersion for both micronucleus and chromosomal aberration studies. The results of the study showed that, the control group treated with physiological saline induced an average of 0.029% micronucleated cells (PCE) where as the physiological saline extract of the dental materials induced an average of 0.042% micronucleated cell (PCE). This was not statistically significant when compared to control group. The positive control cyclophosphamide induced (5.925%) a statistically significant ($P < 0.001$) percentage of micronucleated cells [Table 2, Figure 1]. Table 3 shows that the physiological saline extract of dental materials (test) induced a total of 0.029% breaks and 0.017% gaps, where as the physiological saline alone (control) induced a total of 0.004% breaks and 0.008% of gaps. Similarly, positive control cyclophosphamide induced 7.667% of breaks and 1.325% of gaps. The positive control cyclophosphamide induced a statistically significant percentage of breaks and gaps [Figure 2].

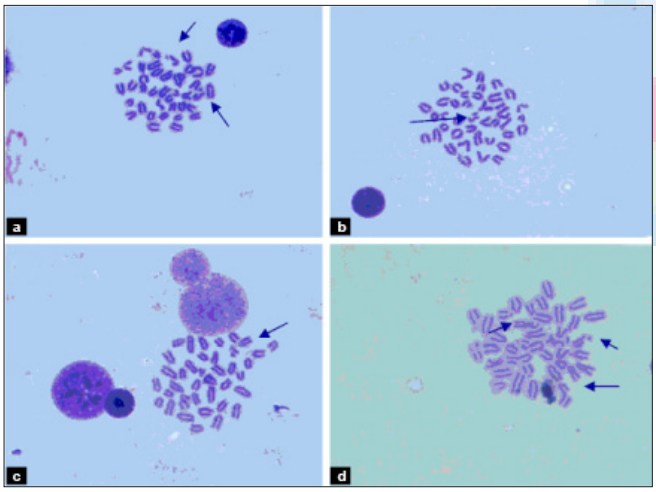


Figure 1: Polychromatic erythrocytes with micronuclei induced by cyclophosphamide., Arrow indicated PCE with micronuclei (a,b), NCE with micronuclei (c) and NCE (d), PCE: p olychromatic erythrocytes, NCE: normochromatic erythrocytes.

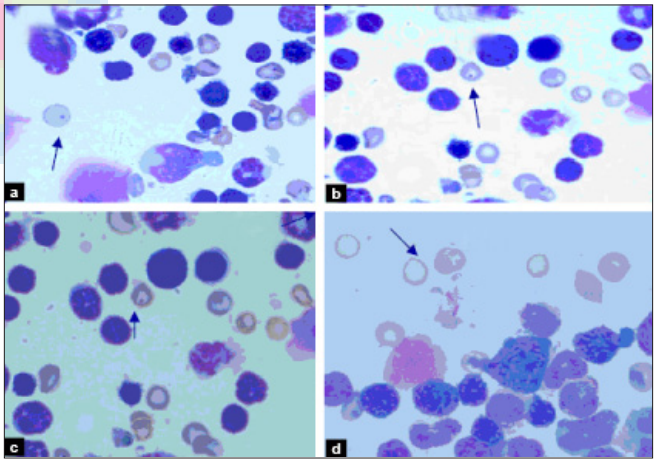


Figure 2: Chromosomal aberrations induced by cyclophosphamide. Arrow indicated chrmoatid breaks (a-c) and dicentric (d).

Table 2: Effect of physiological saline extract of ORMO-48 on micronuclei

Group	No. of animals	Dose	Total polychromatic erythrocytes counted = 2000					
			#PCE with MN (%)	#ICE with MN (%)	PCE with MN (%) Mean± SE	NCE with MN (%) Mean±SE	PCE+NCE cells with MN%) Mean±SE	PCE/NCE ratio Mean±SE
I (PS)	6	50 ml/kg	0.667	0	0.029 0.025	0	0.026 0.020	2.577 0.179
II (DM)	6	50 ml /kg	0.833	0.167	0.042 0.020	0.032 0.032	0.047 0.019	2.593 0.294
111 (CP)	6	20 mg/kg	118.83	4.83	5.925[a] 0.432	0.413 0.265	4.083[a] 0.338	2.107[c] 0.187

PCE: Polychromatic erythrocytes, NCE: Normochromatic erythrocytes, PS: Physiological saline, MN: Micronucleated cells, CP: Cyclophospahamide, #: average of six animals, DM: Dental materials, statistically significant to PS [a: $P < 0.001$, c: $P < 0.05$].

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Table 3: Effect of physiological saline extract of ORMO-48 on chromosomes

Group	No. of animals	Dose	Total no. of metaphase counted=100 (all values are Mean ±SE)					
			Clumatid breaks (%)	Chromatid gaps (%)	Chromosome breaks (%)	Chromosome gaps (%)	Total breaks (%)	Total gaps (%)
IV (PS)	6	50 ml/kg	0.004 0.004	0	0	0.008 0.008	0.004 0.004	0.008 0.008
V (CP)	6	20 mg/kg	7.500[c] 2.721	1.021[a] 0.115	0.167[b] 0.048	0.304[b] 0.085	7.667[c] 2.720	1.325[a] 0.090
VI (DM)	6	50 ml/kg	0.008 0.005	0.008 0.045	0.021 0.016	0.008 0.008	0.029 0.015	0.017 0.008

PS: Physiological saline, CP: Cyclophospahamide, DM: Dental materials, statistically significant to PS [a: $P < 0.001$, b: $P < 0.01$, c: $P < 0.05$].

Establishing toxicity of medical devices and their materials is of vital importance in ensuring product safety.[16] Biomaterials must be evaluated by consistent and specific cytogenetic studies. These tests are to be carried out essentially before a new product is used clinically.[10] Genotoxicity testing is one of the tests used to evaluate this fundamentally important issue and is generally used for hazard identification with respect to DNA damage. These damages can be manifested in the form of gene mutation, structural chromosomal aberration, recombination, numerical changes.[1,15,17-19] The result of the micronucleus study indicated that, the percentage of micronucleated PCE and NCE induced by the dental material (extract) was well comparable with control group, where as the positive control induced significantly high ($P < 0.001$) micronucleated PCE when compared to control. The PCE and NCE ratio of the treated group was similar to that of control group. This confirms that the physiological saline extract of dental material had no effect on the proliferative activity of bone marrow. The chromosomal anomalies such as chromatid/chromosomal breaks, centric rings, exchanges, dicentric, and acentric fragments were evaluated. The result of the eight chromosomal aberration test showed that the anomalies in the treated group (test) were similar to control group; however, significant anomalies were observed in the cyclophosphamide treated group ($P < 0.001$). Hence, the present study concluded that the indigenously developed dental material, ORMO - 48 failed to induce micronuclei and chromosomal anomalies in bone marrow of Swiss albino mice and confirmed that it was non-genotoxic at our laboratory conditions.

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