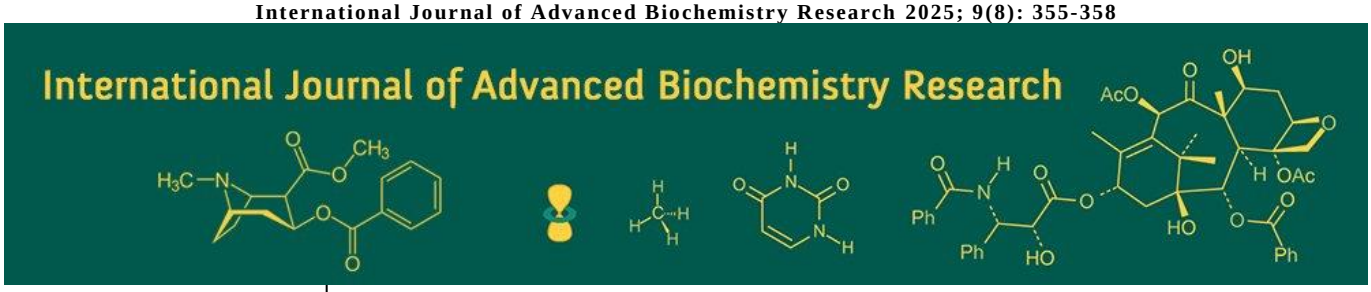

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Ajit Kumar Naik
CVSc & AH, OUAT,
Bhubaneswar, Odisha, India

Sitesh Kumar Mohapatra
CVSc & AH, OUAT,
Bhubaneswar, Odisha, India

Jeevan Ranjan Das
CVSc & AH, OUAT,
Bhubaneswar, Odisha, India

Corresponding Author:
Ajit Kumar Naik
CVSc & AH, OUAT,
Bhubaneswar, Odisha, India

Formalin induced genotoxicity in rats and its amelioration with Eugenol and curcumin

Ajit Kumar Naik, Sitesh Kumar Mohapatra and Jeevan Ranjan Das

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Abstract
Not a single *in vitro* assessment is able to detect the type of damage to cells; a number of tests are performed, like gene mutation, test for aberration of chromosomes, bone marrow MNT, and comet assay. The study was done with 6-10-week-old Wistar rats (n = 54), both male and female, weighing 200-250 grams, and divided into 9 groups with 6 animals in each group. Formalin at the rate of 40 mg/kg was administered for 10 days. Cyclophosphamide at 20 mg/kg was used as a positive control for genotoxicity and was administered 24 hours before sacrificing the experimental animals. Eugenol was used at a dose of 50 mg/kg body weight. The present work was intended to assess the genotoxicity of formalin in Wistar rats, and Curcumin (50 mg/kg BW) was supplemented orally to evaluate its ameliorative effect on the induced toxicity. After 21 days of experimentation, blood was collected and the rats were sacrificed. Bone marrow flushing was taken for assessing chromosomal aberration and micronuclei assay. From a series of genotoxic tests, it was found that oral administration of 40 mg/kg BW of formalin induced a significant level (p<0.05) of genotoxicity compared to the negative control, but it was not to the extent of damage caused by Cyclophosphamide at 20 mg/kg (positive control). The ameliorative effect of both Eugenol and Curcumin resulted in improving the damaging parameters to a minimal extent, which was found to be non-significant.

Keywords: Formalin, genotoxicity, Eugenol, curcumin, comet assay, rat

Introduction
Some substancs caus genotoxicity by cellular damage. To assess genotoxicity, researchers research look for cellular and DNA damage. The major genotoxicity are mutations of genes, aberrations in chromosome and deffects in DNA. No single method is perfect to assess the cellular and DNA damage, different methods are employed. They are 1. One of the best test to observe Genotoxicity is to measure and test DNA damage in single cell which show both qualitative and quantitative measurement (Comet Assay), 2. Bone marrow micronucleus test (MNT) is the most accurate test for Genotoxicity with simultaneous assessment of aberration in chromosome, 3. Gene mutation and chromosomal aberration tests are for identification of actual lesions in the DNA molecule.

Materials and Methods
54 wistarrats, both male and female were grouped into 9 different groups consisting 6 animals in each groups. Group I, VIII, IX were administered with 1ml NS, 50 mg/kg BW, Eugenol and 50 mg/kg BW Curcumin orally respectively. Group II, IV, V rats were administered Cyclophosphamide (CYP) @ 20 mg/kg BW in IP route, CYP with Eugenol, CYP with Curcumin as the above mentioned dose respectively. Group III, VI, VIIratswerefed1% Formalin, Formalin along with Eugenol and Formalin along with Curcumin respectively oral lavage It was observed that genotoxicity developed in different groups and the amelioration effects were calculated through data from Single Gel Electrophoresis (Comet Assay), Bone marrow micronucleus test (MNT), chromosomal aberration tests in bone marrow cells by using Malhiand Grover (1987) [13] and Chauhan *et al.* (2000) [4]. Micronucleus assay in bone marrow cells was done by Hayashi *et al.* (1983) and Chauhan *et al.* (2000) [4]. Single cell gel electrophoresis (SCGE) / Comet assay was performed in cattle blood using Singh *et al.* (1988) [16].

Results

Average number of micronuclei (MN) per 2000 PCE was significantly highest in Gr-II (Table 1) as compared to other groups. The micronuclei seen in Gr-I, Gr-VIII, and Gr-IX were on the lower side, and no significant difference was observed. In the formalin-treated group, this value was 3.93±0.36, which was significantly higher than both of the control groups, whereas the Gr-VI and Gr-VII values showed a decreasing trend from Gr-III, which was not significant. Similar trends were also seen for Gr-II in comparison with Gr-IV and Gr-values

Table 1: Mean micronuclei/2000 polychromatic erythrocytes (PCE), data expressed as (Mean ± SEM)

Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI	Gr-VII	Gr-VIII	Gr-IX
1.28^{a}±0.13	10.76^{d}±1.82	3.93^{bc}±0.36	10.56^{d}±1.67	10.32^{d}±1.41	3.64^{bc}±0.27	3.22^{b}±0.34	1.25^{a}±0.14	1.22^{a}±0.18

N.B.: Mean bearing different superscript differs significantly at 5% level (p<0.05), n = 6 in each group. One way ANOVA was done to analyze the data using Statistical Analysis System (SAS).

PCE (mean) per 200 total erythrocytes (TE) in bone marrow cells of rats was significantly lowest (59.33±3.32) in Gr-II, i.e., Cyclophosphamide-treated rats. The administration of Eugenol (Gr-IV) and Curcumin (Gr-V) resulted in improving the condition. The mean PCE values of Gr-I, Gr-VIII, and Gr-IX were significantly higher than those of other groups. In Gr-III rats, this value was 92.33±3.51, which was significantly different from both positive and negative controls. The values of Gr-VI and Gr-VII showed an improving tendency compared to Gr-III, which was non-significant (Table 2).

Table 2: Mean polychromatic erythrocytes (PCE) /200 total erythrocytes (TE), data expressed as (Mean ± SEM)

Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI	Gr-VII	Gr-VIII	Gr-IX
109.83^{d}±3.55	59.33^{a}±3.32	92.33^{c}±3.51	66.17^{ab}±2.17	67.83^{b}±2.96	96.83^{c}±1.58	99.17^{c}±1.80	112.33^{d}±2.30	114.17^{d}±1.62

N.B.: Mean values bearing different superscripts differ significantly at the 5% level (p<0.05), n = 6 in each group. One-way ANOVA was used to analyze the data using the Statistical Analysis System (SAS).

Chromosomal aberration assay

The mean structural chromosomal aberration in different groups from Gr-I to Gr-IX was observed as 1.16±0.12, 10.65±1.09, 4.45±0.23, 10.34±1.04, 10.11±0.92, 4.22±0.23, 4.10±0.32, 1.13±0.15, and 1.10±0.11, respectively. The highest pulverization was seen in Gr-II rats, while the least was observed in Gr-I, Gr-VIII, and Gr-IX. A significantly higher value was seen in the formalin-treated group (Gr-III) compared to the negative control group. Administration of Eugenol and Curcumin reduced the chromosomal aberration (CA) value, though the reduction was non-significant (Table 3).

Table 3: Mean structural chromosomal aberration in different groups, data expressed as (Mean ± SEM)

Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI	Gr-VII	Gr-VIII	Gr-IX
1.28^{a}±0.13	10.76^{d}±1.82	3.93^{bc}±0.36	10.56^{d}±1.67	10.32^{d}±1.41	3.64^{bc}±0.27	3.22^{b}±0.34	1.25^{a}±0.14	1.22^{a}±0.18

N.B.: Mean values bearing different superscripts differ significantly at the 5% level (p<0.05), n = 6 in each group. One-way ANOVA was used to analyze the data using the Statistical Analysis System (SAS).

Comet assay

The value of OTM per cell in Gr-II (2.37±0.017) rats was significantly highest from all other groups. The lowest values of OTM were seen in Gr-1, Gr-VIII & Gr-IX. In Formalin treated rats (Gr-III), thevalue0.63±0.052 was observed which differ significantly from both positive and negative control groups i.e. Gr I and Gr II. In Gr VI and VII this OTM value were observed 0.60±.009 and 0.59±0.022 respectively which was slightly lower than the Gr-III data. The data of OTM of Gr-IV and Gr-V showed slight decrease from Gr-II data which was non-significant (Table.4)

Table 4: Mean OTM (in µm) per cell in different groups, data expressed as (Mean ± SEM)

Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI	Gr-VII	Gr-VIII	Gr-IX
0.05^{a}±0.002	2.37^{c}±0.017	0.63^{b}±0.052	2.36^{c}±0.037	2.32^{c}±0.023	0.60^{b}±0.009	0.59^{b}±0.022	0.05^{a}±0.002	0.04^{a}±0.004

N.B.: Mean values bearing different superscripts differ significantly at the 5% level (p<0.05), n = 6 in each group. One-way ANOVA was used to analyze the data using the Statistical Analysis System (SAS).

Discussion

Genotoxicity by substances and their amelioration by eugenol and surcumin were studied.

Bone marrow micronucleus test (MNT) is a reliable genotoxic test that can predict 100% genotoxic potential (Rao *et al.*, 2005). In the present study, the micronucleus assay revealed that the average number of micronuclei per 2000 PCE in formalin-treated rats (Gr-III) was significantly higher than that of the negative control group (Gr-I). However, this value was significantly lower than that of the positive control group. The increase in the number of micronuclei following formalin exposure is supported by the findings of Mert *et al.* (2014) [15] in *Nile tilapia*, Heddle *et al.* (1981) [7], and Ma *et al.* (1985) [14] in mouse peripheral erythrocytes, as well as Norppa *et al.* (1985) [17] in human lymphocytes and Chinese hamster ovary cells. In contrast, Speit *et al.* (2006) [18] observed no significant increase in micronuclei in the nasal mucosa of rats at different doses of formalin.

The administration of Eugenol (Gr-VI) and Curcumin (Gr-VII) in rats previously exposed to formalin caused a decrease in the MNT value, although this reduction was not statistically significant. The slight decrease observed may be attributed to the ameliorative effect of these herbal

treatments. Similar protective effects were reported with citrus and ellagic acid by Hosseinimehr and Karami (2005) [8], respectively, in studies on Cyclophosphamide-induced genotoxicity.

In the positive control group (Gr-II), a significantly highest MNT value per 2000 PCE was observed, which aligns with the findings of Samarth *et al.* (2018) [19]. The mean number of PCE per 200 total erythrocytes in bone marrow cells of rats was significantly lowest in Gr-II, i.e., Cyclophosphamide-treated rats. This significant reduction may be due to the known genotoxic effects of Cyclophosphamide. The administration of Eugenol (Gr-IV) and Curcumin (Gr-V) led to an improvement in this value, likely due to the ameliorative effects of these traditional therapeutic agents.

The mean PCE values of Gr-I, Gr-VIII, and Gr-IX were significantly higher compared to other groups. In Gr-III rats, this value was 92.33±3.51, which was significantly different from both the positive and negative control groups. The values in Gr-VI and Gr-VII showed improvement compared to Gr-III, although the differences were not statistically significant.

Different structural chromosomal aberrations such as breaks, caps, deletions, exchanges, and pulverization are detected in bone marrow metaphase analysis. These genetic damages are directly or indirectly used to assess the genotoxic effects of certain toxic chemicals (Jena *et al.*, 2002) [9]. In the present study, the highest level of damage (pulverization) was observed in Gr-II rats, whereas the least damage was seen in Gr-I, Gr-VIII, and Gr-IX. A significantly higher value was observed in the formalin-administered group (Gr-III) compared to the negative control group.

This elevated chromosomal aberration was also reported by Cortes *et al.* (1986) [5] in the root meristem of *Allium cepa*, Rieger and Michaelis (1960) [20] in the primary roots of *Vicia faba*, Ma *et al.* (1985) [14] in mouse peripheral erythrocytes, Norppa *et al.* (1985) [17] in Chinese hamster ovary cells, and Nocentini *et al.* (1980) [21] in monkey kidney cells. Bezerra *et al.* (2017) [2] observed the antioxidant, anti-carcinogenic, cytotoxic, and antitumor properties of Eugenol.

The administration of Eugenol and Curcumin in both formalin-and cyclophosphamide-administered groups caused a reduction in chromosomal aberration (CA) values, although this reduction was not statistically significant. The decrease in CA values may be attributed to the ayurvedic and therapeutic properties of these compounds. The findings of Fanqiun and Zhijia (2017) [10], Bezerra *et al.* (2017) [2], and Binu *et al.* (2018) [3] on Eugenol, and Panda (2017) [22] on the anticancer properties of Curcumin, support these observations.

The mean value of OTM (Olive Tail Moment) per cell in Gr-II animals was significantly higher than in all other groups. The lowest OTM values were observed in Gr-I, Gr-VIII, and Gr-IX. In formalin-administered rats (Gr-III), a value of 0.63±0.052 was recorded, which was significantly different from the negative control group. This increase in tail moment and tail DNA percentage is attributed to the damaging properties of formalin. Similar findings were reported by Lu *et al.* (2005) [12], Kumarave *et al.* (2009) [11], and Borkotoky *et al.* (2014) [1] for Nimesulide.

In Gr-VI and Gr-VII, the OTM values were 0.60±0.009 and 0.59±0.022, respectively, which were slightly lower than the Gr-III value. The OTM data for Gr-IV and Gr-V showed a slight decrease compared to the Gr-II group, though the differences were non-significant. The reports of Fanqiun and Zhijia (2017) [10], Bezerra *et al.* (2017) [2], and Binu *et al.* (2018) [3] on Eugenol, and Panda (2017) [22] on the anticancer properties of Curcumin, are in agreement with the present findings.

Declaration of Interest

This was part masters programme of Dr. Sitesh Kumar Mohapatra, an M.V.Sc scholar. The authors declare no conflict of interest.

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