Chemical and Pharmaceutical Bulletin

Regular Article

The Protective Effect of Naringenin on Oxaliplatin-Induced Genotoxicity in Mice

Majid A Ganaie*1, Basit L Jan2, Tajdar H Khan1, Khalid M Alharthy1, Ishfaq A Sheikh3

1Department of Pharmacology, College of Pharmacy, Prince Sattan Bin Abdulaziz University, Al-Kharj, Saudi Arabia

2Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

3King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

*Corresponding author:
Dr Majid A Ganaie,
Department of Pharmacology,
College of Pharmacy,
Prince Sattam Bin Abdulaziz University,
P.O. Box 173, Al-Kharj, 11942,
Saudi Arabia.
Tel.: 00966-11-5886036
E-mail: majidsays@gmail.com
SUMMARY

Oxaliplatin is a third generation platinum based anti-cancer drug used against various human malignancies but displays genotoxic properties against normal cells. Naringenin is a naturally occurring bioflavonoid that possesses anti-oxidant properties and has protective effects against DNA damage. The aim of this study is to examine the protective effects of naringenin on oxaliplatin-induced DNA damage in mice. A total of 50, male BALB/c mice were randomly divided equally into five groups. Oxaliplatin toxicity was induced by a single dose (7 mg/kg b.w.) injection (i.p.) of oxaliplatin. Naringenin was given orally for ten consecutive days at two doses, 20 mg/kg b.w. (dose I) and 40 mg/kg b.w. (dose II), to group I and group II, respectively. On the tenth day of the experiment, animals in groups III, IV, and V were given a single i.p. injection of oxaliplatin (7 mg/kg b.w.). All the animals were sacrificed 24 h after oxaliplatin treatment. The extent of genotoxicity was assessed by multiple genotoxicity assays (8-Hydroxydeoxyguanosine marker, comet, micronucleus and chromosomal aberration assays, oxidative stress-marker Glutathione evaluation) in order to determine diverse kinds of DNA damage. The results indicated that naringenin administration significantly reduced the DNA damage induced by oxaliplatin possibly due to its strong anti-oxidant properties. The results suggest that naringenin is a potential candidate for future development as a chemoprotective agent against chemotherapy associated complications.

Keywords: Oxaliplatin, Naringenin, Genotoxicity, DNA damage, chemoprotective agent
INTRODUCTION

With advent in research and technology, development of novel anti-cancer drugs has begun to offer a lot of hope for cancer patients around the world. However, most of the current developed anti-cancer drugs have off target effects, and are unable to differentiate between cancer and normal cells. There have been reports that have revealed the genotoxic and carcinogenic effects of these drugs. Thus, there has been wide-ranging research to develop efficacious and less harmful anti-cancer drugs or use natural anti-oxidants that could minimize current anti-cancer drugs induced toxicities.\(^1\)

A third generation diaminocyclohexane (DACH) platinum-based anti-cancer drug, oxaliplatin, currently used for colorectal,\(^2\) pancreatic and ovarian cancer treatment.\(^3-4\) Additionally, it has a clinical activity and accepted off-label indications in other types of cancer, such as esophageal, gastric and refractory testicular cancers.\(^5-9\) However, the use of oxaliplatin is associated with severe complications and a number of toxicities such as neurological, hematologic and gastrointestinal toxicities.\(^10-11\) Symptoms unfold early in treatment, usually during the initial cycles and appear frequently with each round of treatment.\(^12\) Approximately 25 to 30% patients develop chronic neuropathy after continued oxaliplatin treatment due to pooled toxicity which results in pain and loss of sensation.\(^13\) In addition, it is also associated with rare cases of ototoxicity\(^14-15\) and hepatotoxicity.\(^16-17\) It has also been reported that highly toxic DNA lesions are caused by oxaliplatin based therapy at the cellular level\(^18\) and may result in DNA damage and therefore higher risk for development of secondary malignancies.\(^19\) In fact, there have been a few cases of secondary acute leukemia reported with oxaliplatin-based therapy.\(^20-22\) These oxaliplatin-induced toxicities limit its utilization in medical oncology as a powerful chemotherapeutic agent. Thus, finding a treatment that significantly restricts or mitigates oxaliplatin-induced genotoxicity and DNA damage of normal cells is vital for the enhancement of therapeutic outcome of oxaliplatin.
Flavonoids are naturally occurring phenolic compounds with a diverse range of bioactivities. Naringenin (4,5,7-trihydroxyflavanone) (NG), a predominant flavanone in citrus fruits like oranges and grape-fruit, is a polyphenolic compound comprising of two benzene rings connected together with a heterocyclic pyrone ring. Several studies have reported that NG has many protective effects including anti-inflammatory, anti-oxidant and anti-carcinogenic and neuroprotective effects. It has also been reported that NG possesses anti-proliferative, anti-atherogenic, apoptotic and neuroprotective properties.

The purpose of this study is to demonstrate the beneficial effects, if any, of naringenin pretreatment on oxaliplatin-induced genotoxicity in a small animal study.
MATERIALS AND METHODS

Materials

Oxaliplatin and naringenin were purchased from commercial suppliers (Sigma-Aldrich, Germany). Other reagents and solvents of analytical grade were purchased from local suppliers.

Mice

Animals used in this study were male (BALB/c) mice, 13-15 weeks old, 22-25 g in weight and were acquired from the Animal House Center, Pharmacy College (King Saud University, Riyadh). All the animals were kept in filter-top shoebox cases in a temperature (23-25°C) and humidity (45-55%) controlled environment, with free access to food (standard mouse pellet diet) and water during the experimental period. The study protocols defined in the manuscript were in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee, King Saud University.

Study Design

A total of 50 animals were randomly divided equally into five groups (10 per group). Naringenin was dissolved in DMSO and diluted in saline. Final DMSO concentration was less than 1%. Oxaliplatin toxicity was induced by a single dose (7 mg/kg b.w.) intraperitoneal (i.p.) injection of oxaliplatin (freshly dissolved in saline). This dose has been used in multiple experimental studies reported in the literature \(^{31-33}\) and is comparable to the human patient treatment regimens. The dose of oxaliplatin used in this study was slightly lower than human doses due to the pharmacokinetic differences between humans and small laboratory animals. Group I was designated as vehicle control and was served saline (0.1ml normal saline i.p.)
only. Group II was given naringenin orally (20 mg/kg b.w.) for ten consecutive days. Group III was given a single i.p. injection of oxaliplatin (7 mg/kg b.w.) on the tenth day of the experiment and chosen as positive control. Groups IV and V were given naringenin orally for ten consecutive days at two doses, 20 mg/kg b.w. (dose I) and 40 mg/kg b.w. (dose II), respectively. On the tenth day of the experiment, animals in groups III, IV, and V were given a single i.p. injection of oxaliplatin (7 mg/kg b.w.). All the animals were sacrificed 24 h after oxaliplatin treatment.

**Measurement of 8-OHdG (8-Hydroxydeoxy-guanosine) in Serum**

8-OHdG is one of the most significant indications of DNA damage. Quantification of 8-OHdG was done by OxiSelect Oxidative DNA damage ELISA kit from Cell Bio Labs, SanDiego, CA, USA. The OxiSelect™ Oxidative DNA Damage ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples. The quantity of 8-OHdG in a sample is determined by comparing its absorbance with that of a known 8-OHdG standard curve. The experimental protocol was followed as described in the manufacturer instructions to conduct the experiment.

**Comet assay**

Comet Assay was used to study the potential beneficial effects of NG on oxaliplatin-induced DNA damage following a simplified protocol with slight modifications. Bone marrow cells from femur bones of mice were collected and kept on ice to allow the cells to settle before use. The slides for each animal were prepared within 1 h after sacrifice. The bone marrow cells were diluted with a fresh aliquot of low melting point 0.5% agarose dissolved in Dulbecco’s phosphate buffer at 37°C and placed on microscopic slides pre-
coated with 1% normal melting agarose. Agarose (0.5%; w/v) was used as a top layer over the gel-embedded cells and enclosed with a cover slip for 5–10 minutes at 4°C. For DNA unwinding, gel fixed cells were lysed in a lysing solution (2.5 M NaCl, 10 mM Trizma base, 100 mM disodium EDTA, 8 g/L NaOH, pH 10 using NaOH) at 4°C for 2 h. The slides were put through electrophoresis in an electrophoresis unit for 20 min at 50 V and 400 mA and subsequently stained with 20 µg/mL ethidium bromide and observed under a microscope (Fluorescence, Olympus IX41). For each slide one hundred cells were scored. The slides were examined by a computerized imaging system (Komet 5.5 software, Andor Technology, Belfast, UK) and the amount of DNA damage was evaluated by the obtained tail moment values.

**Micronucleus test (MN test)**

The Micronucleus test (MN test) was used to evaluate the normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs). Mice were sacrificed 24 h after oxaliplatin treatment and bone-marrow cells were flushed out from the femur bones and smears were prepared and stained with May-Grunwald Giemsa as described by Schmidt. From each mouse, 1000 PCEs and 1000 NCEs were observed for micro nucleated erythrocytes (MNPCEs and MNNCEs) at ×1,000 magnification under a microscope. Additionally, the number of PCEs among 1000 NCEs per animal was also noted to estimate bone-marrow suppression and ratio of PCE/NCE was calculated as follows: %PCE = [PCE/(PCE+NCE)]×100.

**Chromosome aberration assay**

Chromosomal aberration assay was carried out according to Preston et al. Animals were injected 0.03% colchicine (2 mg/kg b.w.) intraperitoneally 90 minutes prior to sacrifice.
Bone-marrow cells were obtained from the femur bones of the animals by flushing with 1% sodium citrate solution and subsequently fixed in acetic-acid: methanol (1:3) followed by refrigeration for 30 minutes. Slides were prepared by conventional flame drying, stained with 5% Giemsa stain after 24 h and assessed by observing ≥100 cells per slide under a high resolution microscope.

**Measurement of GSH and GSSG levels**

GSH and GSSG, the reduced and oxidized forms of glutathione, respectively, levels in bone-marrow cells (2×10^6) were quantified by a kinetic assay as described by Rahman et al.,\textsuperscript{37} wherein catalytic quantities of GSH activated a continuous reduction of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) to thionitrobenzoic acid (TNB) at 412 nm. Standard curves of known GSH and GSSH concentrations were used for quantification by parallel estimation for analysis.

**Statistical analysis**

Data obtained were expressed as mean ± SD and analyzed by one-way analysis of variance using GraphPad Prism (version 5.0; GraphPad software Inc., USA) followed by appropriate post-hoc test (Dunnett’s multiple comparison test). Significant difference was indicated if the value was <0.05.
RESULTS

8-OHdG assay

The 8-OHdG results presented in Table 1 indicate a protective effect of naringenin on oxaliplatin-mediated DNA damage. In the oxaliplatin group, 8-OHdG levels were observed to be high (2.91±0.18 ng/L) compared with the control group (1.41±0.21 ng/L). Also, 8-OHdG levels were 2.31±0.43 and 1.81±0.39 ng/L in NG20 and NG40 treatments, respectively. These results suggest that treatment with NG can significantly reduce 8-OHdG levels compared with the control group in a dose dependent manner. Moreover, naringenin prevented oxaliplatin-mediated change of 8-OHdG in a dose-dependent way compared to the oxaliplatin group.

Comet assay

To examine the DNA damage in the bone-marrow cells of the animals, comet assay was carried out and results in terms of average tail length and tail moment are presented in Table 2. Animals in the control group showed large round head and no tail formation. NG-only treatment displayed no genotoxic influence whereas there was a long comet tail formation in a huge proportion of cells in the oxaliplatin-only group signifying DNA damage in these animals. Treatment of animals with NG20 and NG40 led to a statistically significant decline in DNA migration to the tail compared to the oxaliplatin-treated mice.
**Micronucleus test (MN test)**

Results of the micronucleus test are displayed in Table 3, which indicate the prevalence of MNPCes in the oxaliplatin treated group is significantly higher than the control animals. NG treatment alone caused no alteration in the frequency of MNPCE’s compared to the control animals. Pretreatment of animals by NG20 and NG40 to the oxaliplatin administered animals significantly decreased the percentage of MNPCE’s as compared to the oxaliplatin-only treated group.

Additionally, oxaliplatin displayed its cytotoxic effects by significantly inhibiting the percentage of PCE’s which according to our results was restored by pretreatment of animals with NG20 and NG40 in a dose-dependent manner closer to the control group of animals.

**Chromosomal aberration assay (CA assay)**

The results for the CA assay are presented in Table 4. Oxaliplatin treatment shows an increase in the frequency of chromosomal aberrations with a significantly higher proportion of CA at 32.23±2.97% compared to the control group at 8.44±1.01%. Naringenin pretreatment reduced the frequency of CA to 20.67±2.88% and 15.39±2.75% at 20 and 40 mg/kg doses respectively in the oxaliplatin treated animals indicating a significant effect of naringenin treatment.

**GSH and GSSG levels**

The results for this assay are displayed in Table 5. Oxaliplatin injection significantly decreased the GSH levels while increasing GSSG levels in the animals as compared to the control mice. Pretreatment with NG20 and NG40 augmented the GSH level by 29.26% and 44.23% respectively whereas helping in restoring GSSG levels closer to the control levels by 29.16% and 47.61% respectively when compared to the oxaliplatin treated group only.
DISCUSSION

Bone-marrow is the primary site in the body where hematopoietic and stromal stem cells reside and differentiate in an adult cell population. Bone-marrow cells are highly sensitive to clastogenic agents and hence vulnerable to DNA damage. This damage can lead to genetic rearrangements and mutations and therefore can be dangerous particularly in an undifferentiated bone-marrow cell population. The risk of a secondary cancer becomes higher if these cells survive and proliferate. During chemotherapy, the primary goal is to prevent damage to normal cells from the adverse effects of anti-cancer drugs. There have been multiple studies suggesting the use of natural dietary anti-oxidants can influence the development of adverse effects that result from these treatments and in-fact may prevent anti-cancer drug-induced DNA damage.

The genotoxicity of anti-cancer drugs such as oxaliplatin can result in secondary effects in normal cells, usually due to the generation of high levels of free radicals and ROS (reactive oxygen species), leading to oxidative stress. Oxidative stress can alter nucleic acids, proteins and lipids by generating oxidized purines and pyrimidines, single strand breaks and alkali labile site which may result in direct DNA damage. In order to avoid these harmful side-effects, chemotherapeutic drugs are used in combination with some protective or anti-oxidant agents to reduce their toxic effects. The use of antioxidants, such as flavonoids with the ability to reduce the reactivity of free radicals in chemically stable molecules, can decrease oxidative stress and consequently can avoid the oxidative damage to DNA and other molecules.

The pharmacological mechanism of oxaliplatin in cancer treatment is related to its capacity to coordinate with genomic DNA. Despite its success, oxaliplatin has many limitations that include rising resistance and serious side-effects, typically linked with the varying repair processes of nuclear DNA and off-target influences on certain cytoplasmic
constituents. Oxaliplatin interacts with DNA forming adducts resulting in cross-links, a mechanism that induces mutagenicity and may lead to the inhibition of the enzyme DNA polymerase and therefore inhibit transcription, resulting in blockage of the cell cycle and apoptosis. Nucleotide deletion is the main repair process by which DNA adduct’s formed by oxaliplatin are removed. This requires fully functional anti-oxidant and enzyme systems to ensure the efficiency of the repair mechanism.

As mentioned previously, free oxygen radicals may result in serious mutilation at nuclear DNA level. In this context, the extent of genotoxicity is assessed by multiple genotoxicity assays in order to determine diverse kinds of DNA damage. The biomarker, 8-OHdG, released secondary to DNA damage, is regarded as the most significant indicator of DNA damage. Hydroxyl radicals eliminate hydrogen from nucleic acids or react with double bonds, leading to 8-OHdG. There have been various studies suggesting that DNA damage decreases by anti-oxidant augmentation, and our results in the current study support these findings, wherein naringenin utilized its anti-oxidant properties to decrease ROS-mediated 8-OHdG levels indicating the inhibition of oxidative DNA damage.

DNA damage was also examined by comet assay which indicated a rise in the amount of DNA damage in oxaliplatin-treated group of animals as apparent from the significant raise in the comet tail length and tail moment. Comet assay is commonly used to identify initial phases of DNA damage that usually lead to genotoxicity. Genomic damage by oxaliplatin is believed to be its anti-neoplastic action, but the aim should be to reduce this genotoxicity in non-tumor cells. Treatment with NG substantially alleviated oxaliplatin-induced DNA damage in a dose-dependent manner as indicated by the results which illustrates the potent genoprotective efficacy of NG.

Developments of micronucleus (MN) and chromosomal aberration (CA) assays are frequently used as sensitive signs in the mutagenic assays of a drug. A clastogenic agent
may act during cell division and result in chromosomal damage if administered during proliferation.\textsuperscript{59} Oxaliplatin treatment in animals produced high MN frequency in bone-marrow cells suggesting increase in the rate of chromosomal loss or fragmentation during nuclear division. In the CA assay, oxaliplatin treatment produced a high frequency of aberrations, mostly stretching, chromatid break and gap. Treatment with NG significantly minimized the frequency of MN and CA in the bone-marrow cells in a dose-dependent manner as well. These results evidently illustrate the protective role of naringenin against the clastogenic properties of oxaliplatin.

Multiple studies have found that oxaliplatin treatment increases generation of free radicals in the host\textsuperscript{60} and aggregation of free radicals is harmful for cellular genome and other biomolecules, eventually leading to genotoxicity and secondary malignancies.\textsuperscript{61-62} To evaluate if the observed genoprotective effect of naringenin was due to the alteration of the cellular anti-oxidant system, oxidative stress markers like GSH and GSSG were examined. Oxaliplatin administration caused substantial reduction of GSH levels while GSSG levels were increased, which is attributed to the consumption of GSH to confront oxaliplatin induced free radicals. NG treatment restored the GSH and GSSG levels suggesting that the protective role of NG may be facilitated via modification of cellular anti-oxidant system, which confirms previous studies where NG is reported to have anti-oxidant efficacy.\textsuperscript{63}

Findings of these assays demonstrated enhancements in the amount of DNA damage in oxaliplatin-administrated groups while treatment with NG significantly reduced DNA damage in all the assays. These results strongly demonstrate that NG possesses geno-protective efficiency.

Naringenin is a flavonoid considered as a powerful anti-oxidant agent in chemical and biological systems, with a strong cellular anti-oxidant protection. Recently, this substance has gained special attention due to its chemo-protective and radio-protective actions.\textsuperscript{25-30} An
important characteristic of flavonoids, including naringenin, is their anti-oxidant activity, which plays a significant role in the neutralization of free radicals like hydroxyl radicals generated by chemical agents.\textsuperscript{64-65} Consequently, the oxidative damage is lowered in the biological system exerting the chemo-protective effects by elimination of free radicals, inhibition of inflammation, induction of phase II enzymes, suppression of cell proliferation, induction of cell differentiation, inhibition of cell cycle and apoptosis.\textsuperscript{66-67}

In conclusion, this study shows that NG has a preservative role in the mitigation of oxaliplatin-mediated genotoxicity in mice. One possible reason of NG-mediated preservation is that, before oxaliplatin administration, pretreatment with NG could permit inception of free radicals produced by oxaliplatin prior to reaching DNA and causing damage. Moreover, NG-generated induction of DNA repair system might probably happen.\textsuperscript{68} Therefore, NG could be an encouraging chemo-protective agent to tackle oxaliplatin-mediated toxicity and simultaneous treatment failure. It can also be helpful to prevent subsidiary damage in patients in a treatment regime involving oxaliplatin.

\textbf{Acknowledgement}

This project was supported by the Deanship of Scientific Research, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia under the research project number 2016/03/6694.

The authors are grateful to the College of Pharmacy Research Center and the Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia for technical support.

\textbf{Conflict of Interest}

The authors declare no conflict of interest.
References

56. Ince S., Arslan-Acaroz D., Neuwirth O., Demirel H., Denk B., Kucukkurt I., Turkmen R., Food and Chemical Toxicology, 72, 147–153 (2014).
60. Fong C. W., Free Radical Biology and Medicine, 95, 216-229 (2016).
### List of Tables

#### Table 1
**8-OHdG Assay**: Effect of pretreatment with different doses of Naringenin (NG) on 8-OHdG levels induced by Oxaliplatin (OX) in mice bone-marrow cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>(Treatment)</th>
<th>8-OHdG (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>1.41± 0.21</td>
</tr>
<tr>
<td>II</td>
<td>NG</td>
<td>1.29 ± 0.22</td>
</tr>
<tr>
<td>III</td>
<td>Ox</td>
<td>2.91 ± 0.18 *</td>
</tr>
<tr>
<td>IV</td>
<td>Ox + NG20</td>
<td>2.31± 0.43 †</td>
</tr>
<tr>
<td>V</td>
<td>Ox + NG40</td>
<td>1.81± 0.39 †</td>
</tr>
</tbody>
</table>

Data are represented as mean ± Standard Deviation (SD)

* Significantly different from Group I (P<0.05)

† Significantly different from Group III (P<0.05)

#### Table 2
**Comet Assay**: Effect of pretreatment with different doses of Naringenin (NG) on DNA damage induced by Oxaliplatin (OX) in mice bone-marrow cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>(Treatment)</th>
<th>% DNA in tail</th>
<th>Tail Moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>1.751 ± 0.291</td>
<td>2.976 ± 1.188</td>
</tr>
<tr>
<td>II</td>
<td>NG</td>
<td>1.045 ± 0.133</td>
<td>1.455 ± 0.432</td>
</tr>
<tr>
<td>III</td>
<td>Ox</td>
<td>6.375 ± 0.512*</td>
<td>32.534 ± 3.221*</td>
</tr>
<tr>
<td>IV</td>
<td>Ox + NG20</td>
<td>3.213 ± 0.364†</td>
<td>14.442 ± 2.115†</td>
</tr>
<tr>
<td>V</td>
<td>Ox + NG40</td>
<td>2.502 ± 0.011‖</td>
<td>7.145 ± 0.243‖</td>
</tr>
</tbody>
</table>

Data are represented as mean ± Standard Deviation (SD)

* Significantly different from Group I (P<0.05)

† Significantly different from Group III (P<0.05)
Table 3  
**MN Test:** Effect of pretreatment with different doses of Naringenin (NG) on the frequency of micronuclei induced by Oxaliplatin (OX) in mice bone-marrow cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>(Treatment)</th>
<th>% Micronuclei (MNPCE)</th>
<th>%PCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>0.24 ± 0.06</td>
<td>49.1± 2.21</td>
</tr>
<tr>
<td>II</td>
<td>NG</td>
<td>0.21 ± 0.02</td>
<td>46.9 ± 2.44</td>
</tr>
<tr>
<td>III</td>
<td>Ox</td>
<td>1.41 ± 0.07*</td>
<td>39.4 ± 2.66*</td>
</tr>
<tr>
<td>IV</td>
<td>Ox + NG20</td>
<td>0.77 ± 0.05&quot;</td>
<td>43.1 ± 3.95&quot;</td>
</tr>
<tr>
<td>V</td>
<td>Ox + NG40</td>
<td>0.54 ± 0.05&quot;</td>
<td>44.6 ± 2.99&quot;</td>
</tr>
</tbody>
</table>

Data are represented as mean ± Standard Deviation (SD)
* Significantly different from Group I (P<0.05)
# Significantly different from Group III (P<0.05)

Table 4  
**Chromosomal Aberration Assay:** Effect of pretreatment with different doses of Naringenin (NG) on Oxaliplatin (OX)-induced chromosomal aberration in mice bone-marrow cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Chromosomal Aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>8.44 ± 1.01</td>
</tr>
<tr>
<td>II</td>
<td>NG</td>
<td>7.28 ± 0.95</td>
</tr>
<tr>
<td>III</td>
<td>Ox</td>
<td>32.23 ± 2.97*</td>
</tr>
<tr>
<td>IV</td>
<td>Ox + NG20</td>
<td>20.67 ± 2.88&quot;</td>
</tr>
<tr>
<td>V</td>
<td>Ox + NG40</td>
<td>15.39 ± 2.75&quot;</td>
</tr>
</tbody>
</table>

Data are represented as mean ± Standard Deviation (SD)
* Significantly different from Group I (P<0.05)
# Significantly different from Group III (P<0.05)
Table 5
GSH and GSSG Assay: Effect of pretreatment with different doses of Naringenin (NG) on GSH and GSSG levels induced by Oxaliplatin (OX) in mice bone-marrow cells

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>GSH (nM/10*6 cells)</th>
<th>GSSG (nM/10*6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>6.1±0.4</td>
<td>0.72±0.08</td>
</tr>
<tr>
<td>II</td>
<td>NG</td>
<td>6.4±0.3</td>
<td>0.69±0.07</td>
</tr>
<tr>
<td>III</td>
<td>Ox</td>
<td>2.9±0.3*</td>
<td>1.24±0.21*</td>
</tr>
<tr>
<td>IV</td>
<td>Ox + NG20</td>
<td>4.1±0.4&quot;</td>
<td>0.96±0.16&quot;</td>
</tr>
<tr>
<td>V</td>
<td>Ox + NG40</td>
<td>5.2±0.4&quot;</td>
<td>0.84±0.16&quot;</td>
</tr>
</tbody>
</table>

Data are represented as mean ± Standard Deviation (SD)
* Significantly different from Group I (P<0.05)
# Significantly different from Group III (P<0.05)