Original Article
DOI: https://doi.org/10.70749/ijbr.v3i7.1884

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Genoprotective and Antioxidant Effects of *Olea ferruginea* Leaf Extract Through Micronucleus and Antioxidant Assays

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ARTICLE INFO

Keywords: *Olea ferruginea,* Genoprotection, Micronuclei, Antioxidant, Free radicals.

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Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 03-04-2025 Revised: 30-06-2025 Accepted: 15-07-2025 Published: 31-07-2025

ABSTRACT

Olea ferruginea has plenty of phenolic compounds with significant antioxidant potential, but its genoprotective effects against chemotherapeutic drug–induced DNA damage remains unexplored. This study demonstrates the genoprotective and antioxidant activities of *Olea ferruginea* leaf extract against cisplatin-induced genotoxicity in rats, which is supported by its *in vitro* free radical scavenging experiment. Sprague-Dawley rats were used as a negative control, a positive control (cisplatin only 2 mg/kg), and extract treated groups (cisplatin + *Olea ferruginea* extract). The extract treated groups received doses of 25, 50 and 75 mg/kg for 7, 14 and 21 days. The bone marrow genotoxicity was evaluated through a micronucleus experiment, whereas the antioxidant potential was confirmed by using DPPH and H_2O_2 radical scavenging analysis with the extract concentration of 25, 50,100,200 and 500 µg/mL. The obtained results demonstrate that in the positive control group, cisplatin significantly enhanced micronuclei frequency (7.3 ± 2.2 on day 21) as compared to negative controls (1.3 ± 1.2) and the treated group (2.2 ± 0.7). In addition, the micronucleus formation was reduced by treating with *Olea ferruginea* in a dose and time dependent manner. The reduction in micronuclei occurred with the high dose of 75 mg/kg, which resulted in a frequency of 2.2 ± 0.7 on day 21 ($p < 0.0001$). In, *in vitro*, the extract demonstrated concentration-dependent radical scavenging potential with 60.95% for DPPH at 500 µg/mL and 39.79% for H_2O_2 with 500 µg/mL and 59.69 % with 200 µg/mL concentration. These results highlight its performance as a natural chemoprotective and genoprotective candidate and permit supplementary bioactive compound isolation and its mechanistic studies.

INTRODUCTION

Natural products obtained from different plant extracts and their usages in pharmaceutical industries for the treatment of different deteriorating diseases are the need of the era. The usage of natural products has garnered significant attention for its potential genotoxic and genoprotective properties, as evidenced by various *in vitro* and *in vivo* studies. *Olea ferruginea* is rich in bioactive compounds, including polyphenols, flavonoids, and secoiridoids, which have been shown to exhibit antioxidant activities that may mitigate oxidative stress and DNA damage. [1-3]. Micronuclei (MN) are small extranuclear entities that arise in cells when chromosome fragments or complete chromosomes are not incorporated into the daughter nuclei during cell division (mitosis or meiosis).They appear as little, spherical entities that are distinct from the main nucleus and are thought to indicate genomic instability and DNA damage. MN frequency in cells indicate the risk of genotoxicity which leads cancer in cell lines. A decrease in MN frequency suggests that antioxidants or medicinal substances are preventing DNA damage. The micronucleus assay is a common test *(in vitro* or *in vivo*) for finding micronuclei [4-6].

Free radical**s,** e.g., superoxide anion (O_2^-), hydroxyl radical (•OH) and nitric oxide (NO•), are molecules that have one or more unpaired electrons in their outer shell. Reactive Oxygen Species (ROS) are a subset of free radicals that are specifically derived from oxygen. Some ROS are radical ROS (contain unpaired electrons), like superoxide (O_2^-) and hydroxyl radical (•OH), while others are non-radical ROS (still reactive, but no unpaired electron), like hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). Free radicals and ROS frequently destroy key biological structures such as DNA, proteins, and lipids. DPPH (2,2-diphenyl-1-picrylhydrazyl) and H_2O_2

(hydrogen peroxide) assays are used to determine the scavenging activity of the plant extract. [7-9]. The micronuclei study performed over the rat bone marrow was not found in the literature review of the extract of *olea ferruginea* but it is rich in bioactive compounds, which may reduce the cisplatin-inducing genotoxicity. *Olea ferruginea* has been shown to have characteristics that protect the liver and fight cancer, germs, and allergies [10,11], and neuroprotective advantages in a localized brain hypoxia rat model. [12-15]. In addition, the fruit of *Olea ferruginea* has been demonstrated to possess antioxidant properties. [16,17], as well as hepatoprotective characteristics that protect rats from fluoride-induced exposure to lethal levels[18]. More recently, the bark and leaves of *Olea ferruginea* were found to have antibacterial and antioxidant properties. [19].The antioxidant properties of *Olea ferruginea* extracts have been demonstrated through assays such as DPPH radical scavenging, indicating their ability to neutralize free radicals and reduce oxidative damage. [20].

The genotoxic and genoprotective effects of *Olea ferruginea* extract are not revealed in the previous studies with micronucleus detection studies induced by cisplatin. The scavenging assay on *Olea ferruginea* extract has not been thoroughly conducted with various concentrations to establish a connection with its genoprotective and genotoxic effects.

The frequency of the micronuclei in the bone marrow of rats declined significantly by treating with different concentrations of the Olea ferruginea extract along with DNA damage-inducing cisplatin. Treatment with various concentrations of Olea ferruginea directly links its genoprotective effects with its significant antioxidant potential and decrease in micronuclei.

MATERIALS AND METHODS

Experimental material

The methanolic extract of the plant, *Olea ferruginea,* provided by my co-supervisor, Dr. Zafar Ali Shah, Assistant Professor at the University of Swabi, was used as study material for their genoprotective and free radical scavenging activities.

Experimental Design

Healthy young adult (6-10 weeks old at start of treatment) male and female albino Sprague Dawley rats weighing between 195 and 280 g were used as experimental animals for toxicity (genoprotection and genotoxicity) assessment of methanolic extracts of *Olea ferruginea*. Rats were obtained from the animal house of the pharmacy department, University of Peshawar, Peshawar, Khyber Pakhtunkhwa (KP). The experiments were conducted on the animal after obtaining ethical approval from the University Ethical Committee, Department of Pharmacy, University of Peshawar, KP.

Animals were randomly assigned to positive control, negative control, and treatment groups. They were housed in multichamber clean cages under standard environmental conditions of 22°C (±3°C), under a 12 h dark-light cycle, and were allowed free access to drinking water, a standard pellet diet and labitum. Rats were kept in the experimental facility for one week prior to dosing to allow them to acclimatize to laboratory conditions. For *in vitro* free radical scavenging activities, the extract was assessed through DPPH and H_2O_2 scavenging assays through spectrophotometry.

Animal groups and dose level for *in vivo* micronucleus assay

For *in vivo* analysis of the test samples, three different groups (one for negative control only with normal saline, one for positive control with cisplatin 2mg/kg/body weight and one for dose treated) of rats were made for each concentration, i.e., low (25 mg/kg), medium (50 mg/kg), and high (75 mg/kg) body weight of *Olea ferruginea* extract. Each group contained six healthy rats (n=6). For each concentration of test samples, groups were made for 7, 14, and 21days treatment separately. Test samples were injected intraperitoneally into the rat through a 5 ml disposable syringe with 21 G on a daily basis through the treatment regime.

Experimentation

In vivo Micronucleus Assay of *Olea ferruginea* extract in Sprague–Dawley Rats

For the *in vivo* micronucleus assay, we have used the method followed by [21], [22,23] with minor modification in Sprague–Dawley rats. All five groups of the rats, i.e., the controls and the treated, were euthanized in a box with wet cotton of chloroform after each regime of treatment, i.e., after 7, 14 and 24 days. Rat femurs were removed, and bone marrow cells were collected. Briefly, 0.5 mL of 10% neutral formalin was added to the bone marrow suspension for 5 min for fixation, followed by centrifugation (4°C, 1,000 rpm) for 5 min to remove the supernatant. Thereafter, 0.3 mL of 10% neutral formalin was added to the precipitated bone marrow cells for suspension before filtering them through a cell strainer and transferring them to a storage tube. After dropping the fixed suspension of bone marrow cells on a slide, it was stained with 10% Giemsa stain for an hour. After staining, the slides were gently rinsed with phosphate-buffered saline (PBS), air-dried, and examined under a light microscope (400 times magnification; HM-LUX 3, Leitz, Japan) using an oil immersion lens (100×/1.25). A total of 1000 RBCs were examined for finding out the presence of micronuclei in each sample.

Statistical analysis

Micronucleus data were analyzed using Student's t-test. Statistical analysis was performed using SPSS software; p-values of <0.05 were considered to indicate statistical significance. All results were expressed as mean ± SD for six animals in each group.

Free radical Scavenging Assays (FRSA)

The H_2O_2 and DPPH assays are the most frequently used methods for the evaluation of the free radical-scavenging capacity of plant extract. In this research project the radical scavenging activity (RSA) of the extract of *Olea ferruginea* was determined through the hydrogen peroxide (H_2O_2) radical scavenging assay as described by[24,25] and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as described by [26,27] with minor modifications. *Olea ferruginea* was used with 25, 50, 100, 200 and 500 µg/ml. For each concentration, the experiments were repeated in triplicate (n=3). As a

standard, the free radical scavenger L-ascorbic acid was used with the same concentration as the extract, i.e., 25, 50, 75, 150, and 300 µg/ml, for comparison purposes of the test samples with the standard and in between their own different concentrations.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

For H_2O_2 scavenging activity, 0.5 ml of plant extract according to the concentrations mentioned was taken into a test tube, and then 3.4 ml of PBS was added. At last, 0.6 ml (600 µl) of H_2O_2 was added and incubated for 30 minutes at room temperature. All these experiments with each concentration of samples and control were repeated in triplicate (n=3). The absorbance of hydrogen peroxide (H_2O_2) was determined at 230 nm using a UV spectrophotometer (SP-300, Japan) against a blank solution containing phosphate buffer (PBS, PH 7.4) without hydrogen peroxide to avoid background. As a negative control, the solution of H_2O_2 (H_2O_2 + PBS solution) was used for the calculation of the percent radical scavenging activity (RSA) of *Olea ferruginea* extract. L-ascorbic acid was used as a reference (standard) compound for comparison with test samples. The percent radical scavenging activity (RSA) or percent inhibition of samples was calculated by the following equation:

H_2O_2 RSA (%) = (Abs (control) – Abs (sample)/ Abs (control)×100

Where **Abs (control)=** Absorbance of negative control H_2O_2 solution, without any sample (H_2O_2 + PBS solution)

Abs (sample) = Absorbance of test sample (Extract)

% RSA = Percent Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with a violet colour, and the scavenging of DPPH radicals generates a yellow colour. Based on this principle, *Olea ferruginea* extracts have been checked for their free radical scavenging activity. For DPPH scavenging activity, different values of the extract were taken according to the required concentrations and reached 0.5 mL by adding the solvent (methanol). After that, 3.4 ml of methanol was added to each test sample, followed by the addition of 0.6 ml (600 µl) of DPPH (43mM), and the mixture was incubated for 30 minutes at room temperature. All these experiments with each concentration of samples and control were repeated in triplicate (n=3). The absorbance of DPPH was determined at 517 nm using a UV spectrophotometer (SP-300, Japan) against a blank methanol only, without extract of *Olea ferruginea*, to avoid background. As a negative control, the solution of DPPH was used for calculation of percent radical scavenging activity (RSA) of extracts. L-ascorbic acid was used as a reference (standard) compound for comparison with test samples. The percent radical scavenging activity (RSA) or percent inhibition of extract was calculated by the following equation:

DPPH RSA (%) or % inhibition = (Abs (control) – Abs (sample)/ Abs (control)×100

Where **Abs (control)=** Absorbance of negative control DPPH solution, without any sample (DPPH + Methanol solution)

Abs (sample) = Absorbance of test sample (Extract)

% RSA = Percent Radical Scavenging Activity

Calculation of IC_{50} of test sample (extract of *Olea ferruginea*)

IC_{50} (inhibitory concentration) shows the fifty percent inhibition of free radicals by the test samples. Various concentrations of methanolic extracts and compounds were taken for the study and IC_{50} values. IC_{50}, which shows 50% inhibition (50% scavenging), was determined from the regression equation, derived from the graph plotted with the concentration of the samples against the percentage inhibition (scavenging) of the free radical formed in the assay system (DPPH and H_2O_2 assays). All determinations were carried out in triplicate.

RESULTS

***In vivo* effect of *Olea ferruginea* on rat bone marrow via micronucleus assay**

In order to determine the antigenotoxic or genoprotective potential of *Olea ferruginea* via micronucleus assay on rats' bone marrow, the rats were treated with different concentrations of *Olea ferruginea* for 7,14 and 21 days (table 1). The negative control group was given the feed only, while the positive control was exposed to Cisplatin 2mg/kg/day. The rats in the experimental group were exposed to Cisplatin 2mg/kg/day along with 25, 50 and 75 mg/kg/day of *Olea ferruginea*.

Our results indicate that the negative control group, which did not receive any treatment, displays the baseline level of MN without any intervention. The mean values are always low (1.0 to 1.3), which means that MN formation is normal and not very high. In the negative control group, small standard deviations (SD ± 0.7–1.2) indicate that the data points cluster closely around the mean, indicating little variability within this group.

In the positive control group, the genotoxicity is significantly induced by cisplatin by increasing the number of micronuclei (↑ MN). The mean values for this group are always high at all regimes of the treatment, i.e., 5.5, 6.5 and 7.3 for day 7,14 and 21 respectively. The standard deviations for cisplatin for 7,14 and 21 day treatments are (SD ± 1.0) (SD ± 1.2), and (SD ± 2.2), respectively.

The highest effective dosage of *Olea ferruginea* is 75 mg/kg, which produces a statistically significant effect by reducing the number of micronuclei (↓MN) overtime. The dose of 50 mg/kg for 14 days shows a decrease, but with some irregularities. The lowest dose, i.e., 25 mg/kg, performs effectively, but not as well as larger doses. All groups have small standard deviations (SD), which indicates that the data is consistent. The mean value of micronuclei after 21 days treatment reduce significantly from 7.2 to 2.2 with standard deviations (SD ± 2.2 to SD ± 0.7). Thereby, *Olea ferruginea* extract lowers the genotoxicity of cisplatin and has a dose-dependent genoprotective effect.

Table 1: Mean ± SD of MN in rat bone marrow treated with selected concentrations of *Olea ferruginea* for 7, 14 and 21 days.

Groups	Mean ± SD of MN after 7 days	Mean ± SD of MN after 14 days	Mean ± SD of MN after 21 days	Interpretation
Negative control (only feed without treatment)	1.0 ± 0.9	1.2 ± 0.7	1.3 ± 1.2	**Baseline DNA damage.**
Positive control (only Cisplatin 2mg/Kg/day	5.5 ± 1.0	6.2 ± 1.2	7.3 ± 2.2	**Severe DNA damage (worsens over time).**
***Olea ferruginea* 25 mg/kg/day + Cisplatin 2mg/Kg/day**	5.2 ± 1.2	4.0 ± 0.9*	3.2 ± 0.7*	**Significant protection by Day 14.**
***Olea ferruginea* 50 mg/kg/day + Cisplatin 2mg/Kg/day**	4.3 ± 1.4	4.7 ± 1.5	2.5 ± 1.0	**Significant protection by Day 14.**
***Olea ferruginea* 75 mg/kg/day + Cisplatin 2mg/Kg/day**	3.7 ± 0.8*	3.5 ± 1.0*	2.2 ± 0.7*	**Best protection (dose-dependent).**

***SD**, standard deviation; **MN**, micronucleus*
***For 07 days treatment:** The mean difference is significant relative to positive control at *P < 0.05 (One-way ANOVA)*
***For 14 days treatment:** The mean difference is significant relative to positive control at *P = 0.05 (One-way ANOVA)*
***For 21 days treatment:** The mean difference is significant relative to positive control at *P = 0.0001 (One-way ANOVA)*

In the figure below (no. 01), we can see the average frequency of micronuclei (MNs) in rat bone marrow across several treatment groups at 7,14 and 21 days. Negative Control stays low and steady around 1, suggesting just a small amount of DNA damage which is generally possible in the body. The Positive Control (Cisplatin) level rises gradually and reaches approximately 7.3 on day 21, suggesting a high level of genotoxicity. Concentration of 25 mg/kg has a high start but progressively drops, indicating a modest protective effect but concentration of 50 mg/kg indicate moderate protection; initially stable, then a rapid drop by day 21. Treatment with 75 mg/kg shows the greatest decline in MN, indicating the strongest genoprotective effect against cisplatin-induced DNA damage. Conclusively, a whole *Olea ferruginea* extract decreases cisplatin-induced genotoxicity in a dose-dependent manner, with the highest efficacy at 75 mg/kg.

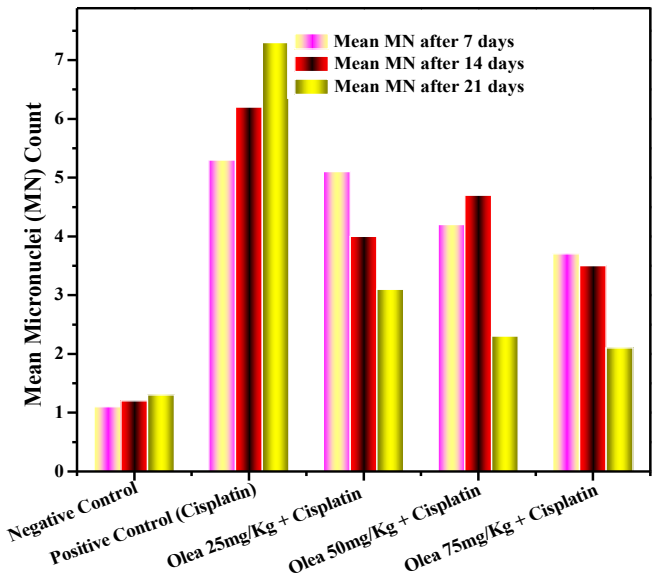

Figure 01: Mean MN in control and treated groups (*In Vivo* treatment with *Olea ferruginea* for 7, 14 and 21 days)

DPPH and H_2O_2 Scavenging Activities of *Olea ferruginea*

Table 2 and Figure 2 show the *in vitro* percent DPPH and H_2O_2 scavenging activities and their IC_{50} values for methanolic extracts of leaves of *Olea ferruginea* at different concentrations performed in triplicate (n=3) for each concentration. The highest value of %RSA by DPPH assay for 500 µg/ml was 60.95% and the lowest, i.e., 18.28%, was for 25 µg/ml. On the other hand, the H_2O_2 scavenging assay shows 14.75% RSA for 25 µg/ml and 39.79% RSA for a concentration of 500 µg/ml. For both the DPPH and H_2O_2 scavenging activities, the *in vitro* percent RSA were concentration dependent, i.e., increased with an increase in concentration of the extract of *Olea ferruginea.*

The IC50 values (µg/ml) of the extract for both the DPPH and H_2O_2 assays were concentration dependent, as IC50 values increased with an increase of the concentrations of the extract. For the DPPH assay, the IC_{50} value was lowest at 1.5 µg/ml for the lowest concentration of 25 µg/ml extract and highest at 20.55 µg/ml for the highest concentration of 500 µg/ml extract. The IC50 values of *Olea ferruginea* for the DPPH assay were lower than the IC_{50} values of the H_2O_2 assay for the same concentrations of the extract.

Table 2: % RSA and IC_{50} of *Olea ferruginea* From DPPH and H_2O_2 Scavenging Assay

Conc. in µg/ml	Average %RSA by DPPH Assay (n=3)	IC_{50} 0f DPPH Assay (µg/ml)	Average %RSA by H_2O_2 Assay (n=3)	IC_{50} of H_2O_2 Assay (µg/ml)
25	18.28	1.5	14.75	1.61
50	27.28	3.81	21.26	4.44
100	43.68	8.27	50.60	10.09
200	53.99	17.20	59.69	21.39
500	60.95	20.55	39.79	55.28

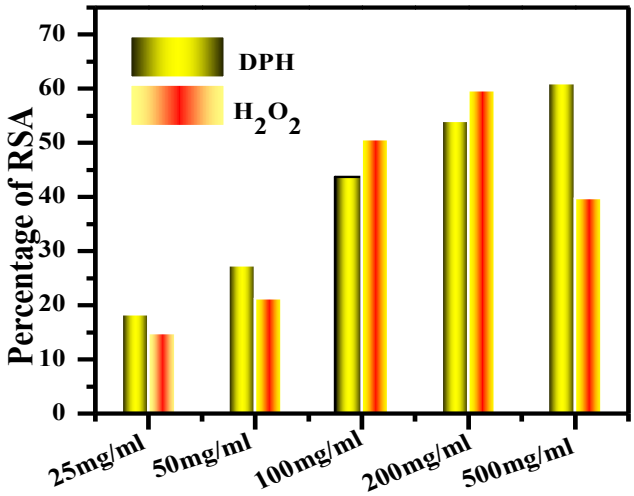

Figure 2: DPPH and H_2O_2 Scavenging Assay of *Olea ferruginea*.

DISCUSSION

The methanolic extract of *Olea ferruginea* was processed and its genoprotective effect investigated through a micronucleus assay against cisplatin- induced DNA damage. The antioxidant effect of the extract was determined through DPPH and H_2O_2 scavenging assays. The extract of *Olea ferruginea* has a lot of multi-effect compounds like Cycloolivil, Ferruginan A, Hydroxytyrosol Acetate, Vanillic Acid, Scopoletin, Isovanillic Acid, Oleuropein, Quercetin, Ligstroside, etc.[28-30].The findings show that *Olea ferruginea* greatly inhibits cisplatin-induced micronuclei development in a dose and time dependent manner, indicating its potential as a natural chemoprotective agent. Cisplatin, a commonly used chemotherapeutic drug, has been shown to cause substantial DNA damage by creating inter and intra strand crosslinks, resulting in chromosomal breakage and micronuclei production [31]. Our results are consistent with earlier publications, as the positive control group (cisplatin-only) showed a progressive increase in MN frequency over 21 days, demonstrating its genotoxic effects. On the other hand, when compared to the cisplatin-only group, co-treatment with *Olea ferruginea* extract at different doses (25, 50, and 75 mg/kg) significantly decreased the frequency of MN. With a significant decrease in MN production by day 21 (2.2 ± 0.7), the highest dose (75 mg/kg) demonstrated the strongest protective effect, suggesting robust genoprotection. This dose-dependent reaction raises the possibility that *Olea ferruginea* contains bioactive substances that can lessen the oxidative DNA damage caused by cisplatin.

Olea ferruginea's antigenotoxic effects may be linked to its high phenolic content, particularly oleuropein, hydroxytyrosol, and flavonoids, which are known antioxidants and free radical scavengers [32,33]. Oxidative stress is a major factor in cisplatin-induced DNA damage and polyphenols can neutralize reactive oxygen species (ROS), preventing chromosomal abnormalities and micronucleus formation [34]. Furthermore, *Olea ferruginea* may improve DNA repair processes or block cisplatin-induced apoptosis in bone marrow cells, as evidenced by the steady decrease in MN frequency over time. Other olive-derived chemicals have been shown to have similar protective properties against chemotherapy-induced damage [35].

The current work examined the *in vitro* antioxidant capacity of *Olea ferruginea* methanolic leaf extract by DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydrogen peroxide (H_2O_2) radical scavenging assays. The findings indicate a concentration-dependent enhancement in radical scavenging activity (RSA), implying that *Olea ferruginea* has considerable antioxidant characteristics, potentially contributing to its previously noted genoprotective activities against cisplatin-induced DNA damage.

The DPPH test is commonly used to assess antioxidants' ability to donate hydrogen [36]. Our study found that *Olea ferruginea* extract has significant DPPH scavenging action, with percentage RSA increasing from 18.28% at 25 µg/mL to 60.95% at 500 µg/mL. The IC_{50} values (concentration required to scavenge 50% of radicals) ranged from 1.5 µg/mL (at 25 µg/mL extract) to 20.55 µg/mL (at 500 µg/mL extract), showing substantial free radical inhibition at lower concentrations. The observed trend aligns with previous studies on *Olea europaea* (olive) leaves, where phenolic compounds such as oleuropein, hydroxytyrosol, and flavonoids were identified as major contributors to antioxidant activity [37]. The lower IC_{50} values at higher dilutions suggest that *Olea ferruginea* may contain highly potent antioxidants that efficiently neutralize DPPH radicals even at minimal concentrations.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) capable of inducing oxidative stress and cellular damage [38]. The H_2O_2 scavenging assay demonstrated that *Olea ferruginea* extract displayed a 14.75% radical scavenging activity (RSA) at 25 µg/mL, which increased to 39.79% at 500 µg/mL, showing a dose-dependent response. Nonetheless, the scavenging effectiveness was typically inferior to that recorded in the DPPH assay, potentially due to variations in radical reactivity and scavenging mechanisms.

Interestingly, the IC_{50} values for H_2O_2 scavenging were greater than those for DPPH (range from 1.61 µg/mL at 25 µg/mL to 55.28 µg/mL at 500 µg/mL), suggesting that *Olea ferruginea* may be more efficient against stable free radicals (like DPPH) than against H_2O_2. This may be attributable to the engagement of distinct antioxidant pathways, including enzymatic (catalase, peroxidase) and non-enzymatic (phenolic) processes [39]. Stronger DPPH scavenging action than H_2O_2 may be due to the extract's phenolic makeup, as polyphenols donate hydrogen atoms to stabilize free radicals [40]. Previous phytochemical studies on Olea species found oleuropein, luteolin, and apigenin derivatives as important antioxidants, possibly explaining their reported radical scavenging properties [41]. Additionally, the concentration dependent increase in RSA supports the idea that *Olea ferruginea* includes numerous bioactive chemicals that work together to neutralize oxidative stress. Other medicinal plant research show that higher extract concentrations

increase antioxidant capability [42].

To the best of our knowledge, herein, we investigate for the first time the *in vivo* genoprotective effects of *Olea ferruginea* leaf extract against cisplatin-induced genotoxicity by using a micronucleus experiment together with an *in vitro* antioxidant assessment.

Olea ferruginea methanolic extract shows a scavenging effect against DPPH radicals. This finding suggests that the extract's primary mechanism of antioxidant action is to neutralize stable free radicals. This research provides credibility for using Olea ferruginea as a natural complement in chemotherapy. The extract's genoprotective benefits, achieved by neutralizing reactive oxygen species (ROS), could help lessen oxidative DNA damage. Future research should identify the specific bioactive compounds responsible for these antioxidant effects.

CONCLUSION

This study presents the first *in vivo* experiment that shows *Olea ferruginea* leaf extract has strong genoprotective effects against cisplatin-induced DNA damage in the bone marrow of rats. The protective outcome was dose and time-dependent. Treatment of rats with 75 mg/kg of extract for 21 days significantly minimizes micronucleus formation in bone marrow as compared to cisplatin alone. The *in vitro* free radical scavenging analysis (both DPPH and H_2O_2) presents significant antioxidant potential. DPPH and H_2O_2 show free radical inhibition with 60.95% and 39.79%, respectively at 500 µg/mL, showing a concentration-dependent trend. To verify its therapeutic potential and safety for humans, more research should focus on isolating and characterizing its active ingredients through standard operating procedures and mechanistic studies. Additionally, clinical validation needs to be done

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