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Genoprotective Effects of Artemisia and Coffee Extracts on Human Lymphocytes from Healthy Individuals and Cancer Patient

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تقييم التأثيرات الواقية من تلف الحمض النووي لمستخلصي الشيح والقهوة في الخلايا اللمفاوية البشرية لدى الأفراد الأصحاء ومرضى سرطان القولون والمستقيم

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Abstract:
cancer remains a major cause of cancer-related mortality worldwide, highlighting the need to investigate potential complementary approaches to cancer management. Natural products have attracted considerable attention for their ability to provide biologically active constituents that may help counteract oxidative stress. The target of this work to study the effects of aqueous Artemisia and coffee extracts, individually and in combination, on oxidative DNA damage in human blood lymphocytes (HPBLs) obtained from healthy donors and patients with colorectal cancer, using the alkaline comet assay. Blood samples were collected from five healthy donors and five patients diagnosed with colorectal cancer. Hydrogen peroxide (H_2O_2; 50 µM) was used to induce oxidative DNA damage, and the effects of Artemisia extract, coffee extract, and their combination were assessed by measuring (% Tail DNA). The results showed different responses to the tested treatments. Artemisia extract alone did not demonstrate a clear protective effect, whereas coffee extract was associated with lower DNA damage than the H_2O_2-treated and Artemisia-treated groups. The combined treatment also reduced DNA damage compared with Artemisia treatment alone. These findings suggest that coffee extract may influence oxidative DNA damage under the experimental conditions, while the effects of the combined treatment require further investigation. Given the limited sample size, larger studies incorporating dose–response assessments and molecular analyses are needed.

Keywords: Artemisia; Coffee; Genotoxicity; Comet assay; DNA damage; Antioxidants; Lymphocytes

الملخص
يُشكّل سرطان القولون والمستقيم تحديًا صحيًا عالميًا، نظرًا إلى مساهمته الكبيرة في معدلات الوفاة الناجمة عن الأمراض السرطانية على مستوى العالم ، مما يبرز الحاجة إلى دراسة استراتيجيات علاجية تكميلية محتملة. وقد حظيت المنتجات الطبيعية باهتمام متزايد بوصفها مصادر للمركبات النشطة حيويًا ذات الخصائص المضادة للأكسدة. هدفت هذه الدراسة إلى تقييم تأثير المستخلصات المائية لنبات Artemisia والقهوة، كلٌّ على حدة وبالاشتراك معًا، في تلف الحمض النووي

التأكسدي في الخلايا اللمفاوية للدم المحيطي البشري المأخوذة من أفراد أصحاء ومرضى مصابين بسرطان القولون والمستقيم، باستخدام اختبار المذنب القلوي.(Alkaline Comet Assay)
جُمعت عينات الدم من خمسة متطوعين أصحاء وخمسة مرضى مشخصين بسرطان القولون والمستقيم. واستُخدم بيروكسيد الهيدروجين (H_2O_2) بتركيز 50 ميكرومولار لإحداث تلف تأكسدي في الحمض النووي، ثم قُيّمت تأثيرات مستخلص Artemisiaومستخلص القهوة والمعاملة المشتركة بينهما من خلال قياس النسبة المئوية للحمض النووي الموجود في ذيل المذنب.(% Tail DNA)
أظهرت النتائج اختلافًا في الاستجابات للمعاملات المختبرة؛ إذ لم يُظهر مستخلص Artemisia منفردًا تأثيرًا وقائيًا واضحًا، في حين ارتبط استخدام مستخلص القهوة بانخفاض تلف الحمض النووي مقارنةً بمجموعتي المعاملة ببيروكسيد الهيدروجين ومستخلص .Artemisia كما أدت المعاملة المشتركة إلى انخفاض تلف الحمض النووي مقارنةً باستخدام مستخلص Artemisiaوحده. وتشير هذه النتائج إلى أن مستخلص القهوة قد يؤثر في مستوى تلف الحمض النووي التأكسدي في ظل الظروف التجريبية المستخدمة، في حين يتطلب تأثير المعاملة المشتركة مزيدًا من البحث والتحقق.
ونظرًا إلى محدودية حجم العينة، هناك حاجة إلى إجراء دراسات إضافية تشمل أعدادًا أكبر من المشاركين، وتقييمات للاستجابة للجرعات المختلفة، وتحليلات جزيئية متخصصة؛ للتحقق من هذه النتائج وتوضيح الآليات المحتملة الكامنة وراءها.

الكلمات المفتاحية: الشيح؛ القهوة؛ السمية الجينية؛ اختبار المذنب؛ تلف الحمض النووي؛ مضادات الأكسدة؛ الخلايا اللمفاوية.

Introduction

Cancer remains a major global health burden and a leading cause of mortality worldwide. Despite significant advances in early detection and therapeutic strategies, its clinical management continues to face major challenges, including drug resistance, systemic toxicity, and tumor recurrence, these limitations have driven increasing interest in the development of alternative or complementary [1].

Many clinically used anticancer agents originate from plant-based compounds. Medicinal plants and commonly consumed dietary products are rich in bioactive constituents capable of modulating key cellular processes such as oxidative stress, inflammation, and signaling pathways involved in cancer progression. Among these, Artemisia species and coffee have garnered significant scientific interest owing to their diverse phytochemical profiles and reported biological activities [2-4].

Phytochemical investigations have demonstrated that Artemisia contains a wide range of bioactive compounds, that contribute to its pharmacological properties. These compounds have attracted considerable interest because of their antioxidant, anti-inflammatory, and potential anticancer properties. Their biological activities are largely associated with their ability to modulate oxidative stress and influence cellular processes involved in the initiation and progression of disease. Coffee, in contrast, represents an important source of dietary bioactive compounds due to its widespread consumption worldwide. Beyond its nutritional and sensory characteristics, coffee contains a complex mixture of phytochemicals. These constituents may exert diverse biological effects, particularly through the regulation of oxidative balance and cellular responses to environmental stressors. Although epidemiological and experimental evidence has suggested potential associations between coffee consumption and a reduced risk of certain cancers, its biological effects are influenced by several factors, including dose, preparation method, and individual susceptibility. Notably, both beneficial and potentially adverse effects have been reported, emphasizing the importance of evaluating its effects on DNA integrity and cellular function. Therefore, investigating the biological activities of Artemisia and coffee extracts, individually and in combination, may provide valuable insight into their potential protective properties and possible interactions at the cellular level [5, 6].

Despite their potential therapeutic benefits, natural compounds may also induce adverse biological effects, including genotoxicity, depending on their concentration and mechanism of action. Genotoxicity refers to the ability of chemical or physical agents to damage genetic material, leading to DNA strand breaks, chromosomal alterations, or mutations, which may contribute to genomic instability or cell death. Therefore, careful evaluation of the genotoxic potential of such compounds is essential prior to their consideration for therapeutic applications [7]. In vitro bioassays using colon cancer cell lines provide a reliable and controlled platform for assessing cytotoxic and genotoxic effects. Among the available techniques, the comet assay is widely recognized as a sensitive and rapid technique for detecting DNA damage. The alkaline comet assay, in particular, is well suited for detecting double-strand DNA breaks and has been extensively applied in genotoxicity studies [8, 9].

Although Artemisia species and coffee are widely consumed and extensively studied for their pharmacological properties, their genotoxic effects on (HPBLs) obtained from healthy and colorectal cancer patient remain insufficiently characterized, particularly in terms of DNA damage [10]. Therefore, this work evaluates and

compare the genotoxic effects of Artemisia and coffee extracts on human colon cancer cells using in vitro bioassays, with a specific focus on DNA damage assessed by the alkaline comet assay, addressing the limited data on their potential DNA-damaging effects and safety profiles.'

Material and methods

Artemisia Extract Preparation

Dried aerial parts of Artemisia were obtained from local sources and authenticated based on morphological characteristics. Before extraction, the plant material was rinsed with distilled water, dried at room temperature, and pulverized to obtain a homogeneous powder. An aqueous extraction was performed by adding 50 g of the powdered material to 500 mL of distilled water. The suspension was maintained at 90–95°C for 3 h with continuous stirring to facilitate the release of water-soluble constituents. After heating, the mixture was left to cool to room temperature. Sodium sulfite (Na_2SO_3; 0.5 g) was then incorporated to limit the oxidation of potentially sensitive phytochemicals. The preparation was kept at 4°C for 4 h before being filtered through Whatman filter paper. The resulting aqueous filtrate was collected for subsequent experimental analyses.

Coffee Extract Preparation

The coffee extract was prepared by combining 2 g of ground coffee with 10 mL of distilled water. The suspension was heated gently below the boiling point to promote the release of water-soluble constituents. Following heating, it was left to cool to room temperature before being filtered through filter paper to obtain the aqueous extract for subsequent experiments. The obtained filtrate was either used immediately or stored at 4°C for short-term use prior to experimental treatment. For cell culture experiments, the extract was diluted to the desired concentrations using RPMI culture medium.

Comet Assay Procedure

Sample Collection and Cell Treatment

Peripheral blood specimens were obtained from five healthy volunteers and five patients diagnosed with colorectal cancer (Tables 1 and 2) through venipuncture after informed consent had been obtained. The collected samples were immediately diluted 1:1 with RPMI-1640 medium containing 10% dimethyl sulfoxide (DMSO). The diluted specimens were transferred into appropriately tubes and stored at 80°C until further use. For the experimental treatments, 100 µL of whole blood was combined with 890 µL of RPMI-1640 medium and 10 µL of the designated treatment solution, yielding a final volume of 1 mL. The treatment solutions consisted of Artemisia extract, coffee extract, or a combination of both extracts. Hydrogen peroxide (50 µM) served as PN, whereas untreated samples were used as NC. All samples were incubated at 37°C for 30 min under standard laboratory conditions before further analysis.

Table 1 Demographic and Clinical Information of Healthy Volunteers

No.	S C	Age	Genus	Smoking
1	SA	48	Female	Non-Smoker
2	AM	54	Male	Smoker
3	AK	49	Female	Non-Smoker
4	EM	33	Female	Non-Smoker
5	MA	45	Male	Smoker

Table 2 Demographic and Clinical Information of Colon Cancer Patients

No.	S C	Age	Genus	Smoking
1	MA	65	Male	Smoker
2	AH	42	Female	Non-Smoker
3	FK	55	Female	Non-Smoker
4	AB	45	Male	Smoker
5	FT	50	Female	Non-Smoker

Comet assay steps

The alkeline comet assay was conducted according to the method of Snegh [8], with minor modifications, to evaluate DNA damage in blood cells. Following treatment, the samples were centrifuged at 3,000 rpm for 5 min, and the recovered cell pellets were gently resuspended in 0.5% (LMP) agarose. Subsequently, 100 µL of the cell–agarose suspension was layered onto microscope slides previously coated with 1% (NMP) agarose. The slides were placed on ice to allow the agarose to solidify. The slides were then immersed in chilled lysis buffer containing 100 mM EDTA, 2.5 M NaCl, 10 mM Tris base, 10% DMSO, and 1% Triton X-100 (pH 10) for at least 1 h to remove cellular membranes and proteins. Following lysis, the slides were transferred to an alkaline electrophoresis buffer containing NaOH and EDTA (pH > 13) to facilitate DNA unwinding. After equilibration at 4°C for 30 min, electrophoresis was performed under cooled conditions at 25 V and 300 mA. Upon completion of electrophoresis, the slides were neutralized with Tris buffer (pH 7.5), rinsed, and stained with ethidium bromide at a concentration of 20 µg/mL. DNA migration patterns were subsequently examined using fluorescence microscopy to assess DNA damage. The main steps of the comet assay are illustrated in Figure 1.

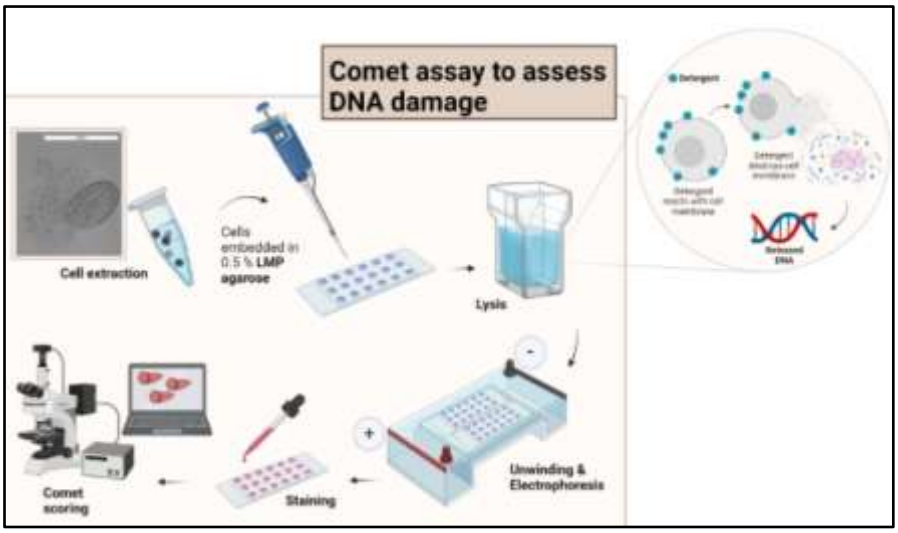


Figure 1: Steps of comet assay

Figure 2 illustrates the laboratory-scale preparation and extraction process for Artemisia plant material. Part (A): Concentrated black extract in an Erlenmeyer flask shows successful dissolution of soluble plant chemicals through boiling. Part (B): A beaker on a magnetic stirrer/hot plate provides continuous stirring and controlled heating to improve solvent–plant interaction while safeguarding sensitive phytochemicals. Part (C): Raw plant material with solvent is covered with aluminium foil on a hot plate to reduce solvent evaporation and protect against airborne contaminants during heating. While, Figure 3: Comet Assay (Single Cell Gel Electrophoresis) setup in the lab and its subsequent procedures. The Figure show how microscope slides are physically handled and prepared during the test procedure.

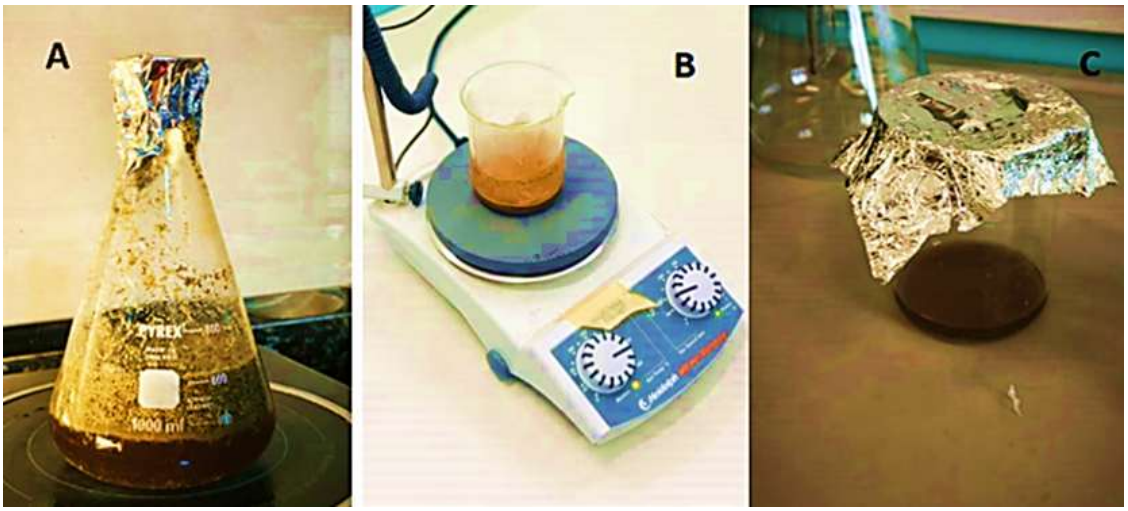


Figure 2: illustrates the laboratory-scale preparation and extraction process for Artemisia plant material

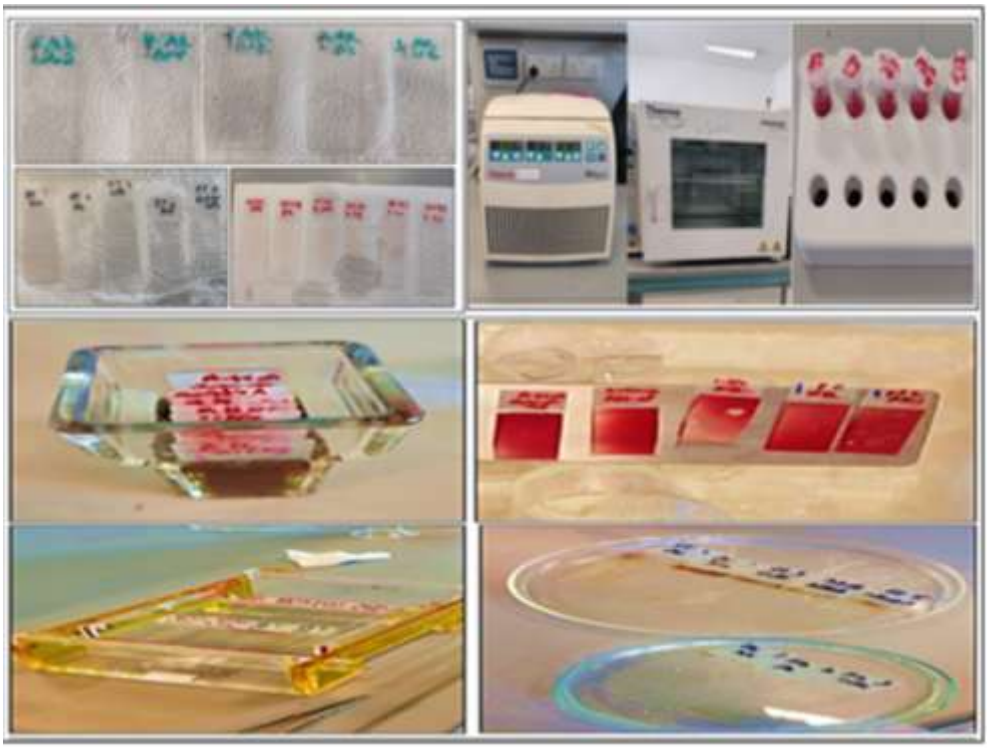


Figure 3: Comet Assay setup in the lab and its subsequent procedures

DNA Damage Assessment

DNA damage was evaluated employing the alkeline comet assay. For each sample, 100 cells were analyzed under double-blind conditions, with all slides coded to minimize potential observer bias. Cell images were captured using a (CCD) camera and analyzed applying a computerized image analysis system. The percentage of Tail DNA and Olive Tail Moment (OTM) were quantified using Komet 6 software (Andor Technology Ltd).

Results

HPLC Analysis of Artemisia Extract

The HPLC chromatogram of the Artemisia extract revealed the presence of several phenolic and flavonoid compounds as can be seen in Figure 4. The major identified constituents included chlorogenic acid, caffeic acid, rutin, quercetin, and gallic acid. Among the detected compounds, chlorogenic acid showed the highest chromatographic peak, indicating that it is one of the predominant phytochemicals present in the extract.

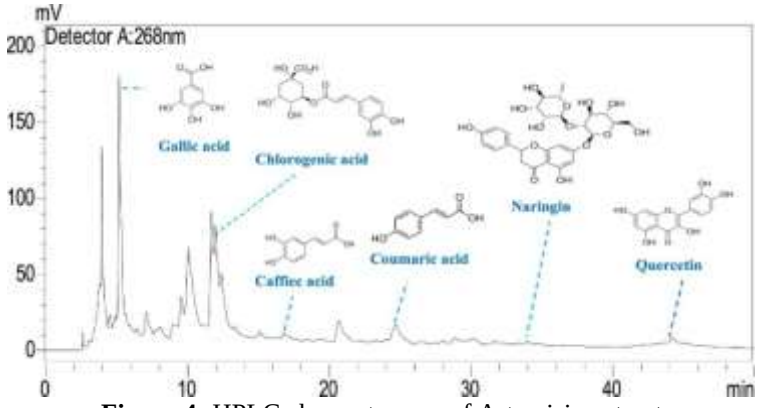


Figure 4: HPLC chromatogram of Artemisia extract

HPLC Analysis of Coffee Extract

Figure 5 shows, the HPLC chromatogram of the coffee extract which, demonstrated the presence of several bioactive compounds. Chlorogenic acid represented the predominant chromatographic peak, followed by caffeine.

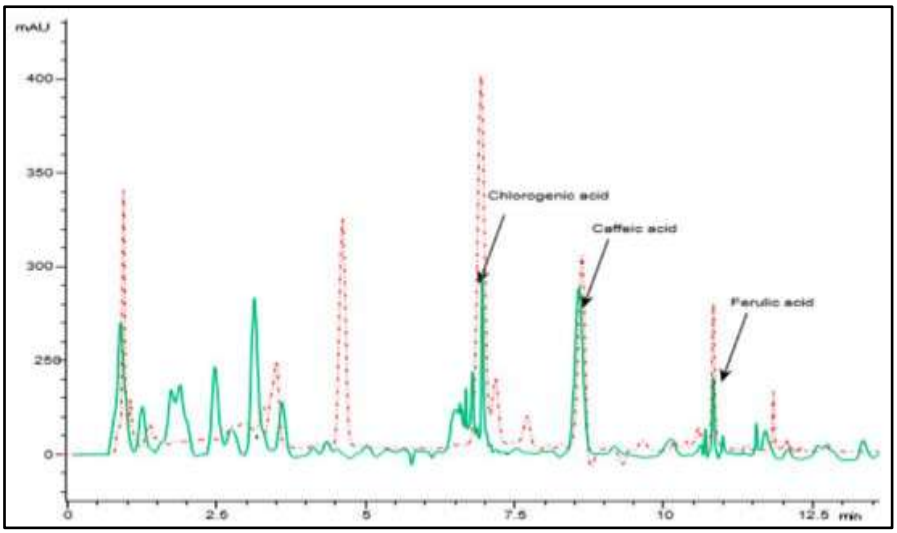


Figure 5: HPLC chromatogram of coffee extract

HPLC analysis revealed several bioactive phenolic acids and flavonoids in both *Artemisia* and coffee extracts, included quercetin, rutin, gallic acid, ferulic acid, and caffeine. These compounds are associated with antioxidant activity and cellular protection against oxidative stress [9].

Chlorogenic acid, detected as a major constituent, along with caffeic acid, gallic acid, quercetin, and rutin, may contribute to reducing oxidative stress and protecting DNA from damage [11]. Their presence may therefore help explain the reduced DNA damage observed in the comet assay following treatment with the extracts. The combined treatment may also benefit from the complementary effects of these phytochemicals. However, research still needed to find the individual contributions of each compound to the observed genoprotective effects.

Comet Assay Analysis

This section presents the findings of the investigation into the potential genoprotective effects of aqueous *Artemisia* and coffee extracts against hydrogen peroxide-induced DNA damage in (HPBLs) obtained from healthy donors and patients with colorectal cancer. DNA damage was evaluated using the alkeline comet assay, with (% Tail DNA) serving as the primary endpoint. The results for the different treatment groups were evaluated in relation to the negative and positive controls to find the range of DNA damage and the potential protective effects of the extracts.

Impact of Treatments on % Tail DNA in Healthy Donors

DNA damage in samples obtained from healthy donors was assessed using the alkaline comet assay, with the percentage of DNA in the comet tail (% Tail DNA) used as an indicator of DNA damage.

DNA damage in samples collected from healthy donors was evaluated utilizing the alkeline comet assay, with the (% Tail DNA) serving as a quantitative measure of DNA damage. ANOVA revealed a highly significant difference in DNA damage levels among the experimental groups ($p < 0.0001$). The negative control (NC) exhibited a mean DNA damage level of 17.89 ± 1.17% Tail DNA (mean ± SEM), whereas the hydrogen peroxide positive control (PC) displayed significantly elevated levels of DNA damage (35.81 ± 2.04%; $p < 0.0001$ vs. NC). Treatment with Artemisia extract resulted in a mean % Tail DNA of 38.28 ± 2.96%, This value was significantly elevated compared with the NC group ($p < 0.0001$), whereas no statistically significant difference was detected relative to the PC group ($p = 0.8879$).

In contrast, coffee treatment yielded a mean % Tail DNA of 15.91 ± 1.79%, which was statistically significant difference was observed from the NC ($p = 0.9422$). However, 2. The coffee-treated group exhibited significantly less DNA damage than either of the two groups of the PC and Artemisia-treated groups ($p < 0.0001$ for both comparisons). Notably, combined treatment with Artemisia and coffee resulted in a mean % Tail DNA of 15.39 ± 1.77%, representing a substantial decrease in DNA damage compared with Artemisia treatment alone ($p < 0.0001$). Furthermore, the combined-treatment group showed no statistically significant difference compared with the NC group ($p = 0.8841$). These findings suggest that coffee co-treatment attenuated the increase in DNA damage associated with Artemisia treatment, restoring the measured DNA damage level to one statistically comparable to that of the negative control.

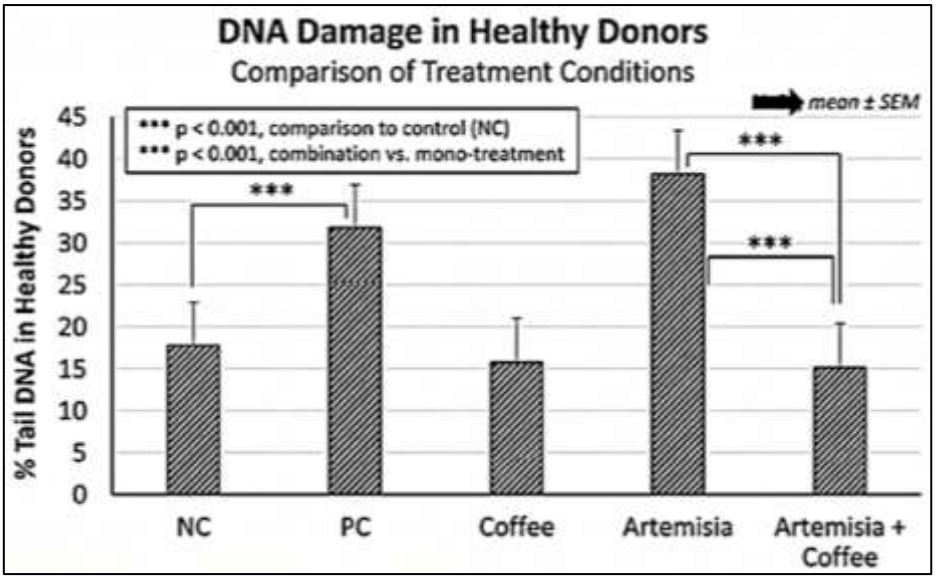


Figure 6: % Tail DNA in Healthy Donors

Impact of Treatments on (% Tail DNA) in Colon Cancer Patient Samples

ANOVA revealed a significant overall difference among the experimental groups ($p < 0.0001$). The negative control (NC) exhibited a mean DNA damage level of 25.10 ± 1.70% Tail DNA (mean ± SEM) for DNA damage in samples obtained from colon cancer patients. The hydrogen peroxide positive control (PC) showed an increased mean % Tail DNA of 38.14 ± 2.30%, which was substantially greater than that of the NC group ($p = 0.0425$).

Artemisia treatment alone resulted in a mean % Tail DNA of 40.46 ± 4.72%, representing a significant increase compared with the NC group ($p = 0.0097$). Nevertheless, the variation among the Artemisia-treated and PC groups no effect was observed ($p = 0.9869$), indicating comparable levels of DNA damage under these treatment conditions. Coffee treatment yielded a mean % Tail DNA of 16.27 ± 3.13%. Although this value was numerically lower than that observed in the NC group, the variation was not statistically significant ($p = 0.3146$). The coffee-treated group showed significantly lower DNA damage than the Artemisia-treated group ($p = 0.0005$) and the PC group ($p = 0.0029$). Overall, these findings indicate that coffee treatment was associated with lower measured DNA damage than either Artemisia treatment alone or the hydrogen peroxide positive control. However, the absence of a statistically significant difference from the NC group does not, by itself, establish equivalence with the baseline level. Accordingly, the outcome should be interpreted in light of the specific treatment comparisons and their corresponding statistical results.

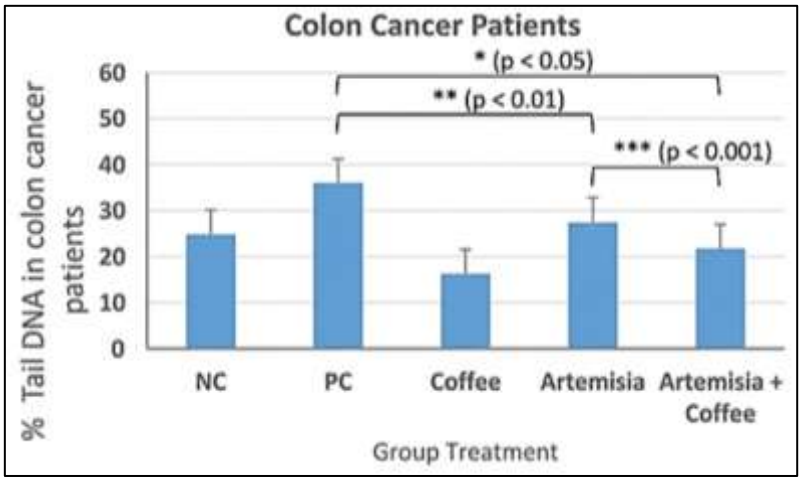


Figure 7: Percentage of Tail DNA in cancer patients under different treatment conditions

Representative Comet Assay Images

Representative comet assay images obtained from healthy donors and cancer patients under different treatment conditions are presented in Figure 8. Cells in the negative control group exhibited compact nuclei with little or no

DNA migration. In contrast, cells treated with hydrogen peroxide (positive control) showed pronounced comet tails, indicating increased DNA damage. Treatment with coffee extract and Artemisia extract reduced the extent of DNA migration compared with the positive control. The combined treatment of Artemisia and coffee also showed reduced comet tail formation, demonstrating a protective effect against H_2O_2-induced DNA damage.

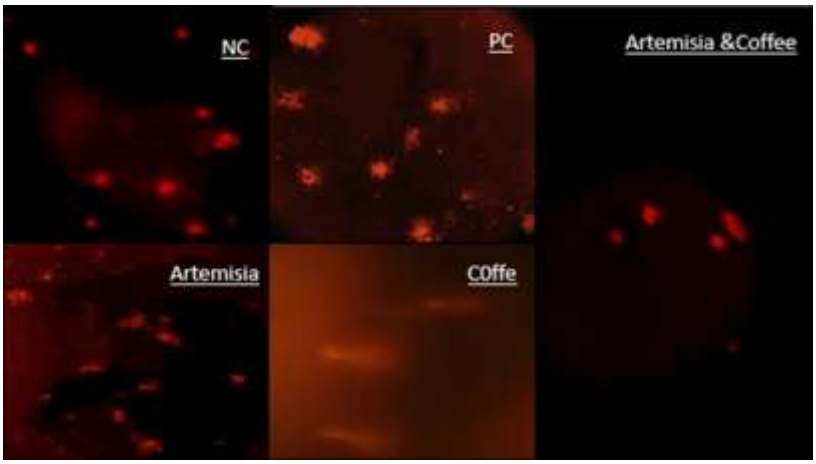


Figure 8: Typical fluorescent photomicrographs of Comet Assay demonstrating nuclear shape and DNA damage under various treatment circumstances

Conclusion

The potential effects of aqueous Artemisia and coffee extracts on oxidative DNA damage in (HPBLs) obtained from healthy donors and patients with colorectal cancer were assessed in this study, using the alkaline comet assay. The findings revealed different responses to the tested treatments. In particular, coffee extract was associated with lower DNA damage than the hydrogen peroxide-treated and Artemisia treated groups, whereas Artemisia extract alone did not demonstrate a clear protective effect under the experimental conditions. The combined treatment also reduced DNA damage compared with Artemisia treatment alone, suggesting that the addition of coffee extract may modify the DNA-damaging response observed with Artemisia. HPLC analysis identified several phenolic and other bioactive compounds in the extracts, including quercetin, rutin, gallic acid, ferulic acid, and caffeine. Some of these compounds have been associated with antioxidant activity and may contribute to the observed biological responses. However, their individual contributions and underlying mechanisms cannot be established from the present results alone. Overall, these findings suggest that coffee extract, either alone or in combination with Artemisia extract, warrants further investigation for its potential to modulate oxidative DNA damage. Nevertheless, the results should be interpreted within the limitations of the present study. Further research involving larger sample sizes, dose–response experiments, additional molecular and biochemical analyses, and appropriate in vivo models is required to validate these observations and clarify the mechanisms involved.

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