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Comparative Cytoprotective Effects of Green and Black Tea Extracts Against Methotrexate Toxicity in In Vitro Models

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A B S T R A C T :

This study aimed to evaluate and compare the cytoprotective effects of aqueous green and black tea extracts against methotrexate-induced toxicity using in vitro brine shrimp (Artemia salina) and Allium cepa models. Green and black tea extracts were prepared from Camellia sinensis leaves and tested for their ability to mitigate methotrexate's cytotoxic effects. In the brine shrimp assay, green tea extract exhibited higher nauplii survival (mean 8.83) compared to black tea (mean 8.33) after 150 minutes, significantly outperforming the methotrexate control (mean 4, p=0.001). In the Allium cepa model, green tea promoted greater root length (12.6 mm) and width (0.99 mm) than black tea (12.1 mm and 0.92 mm) after 96 hours, compared to the methotrexate control (5.7 mm and 0.62 mm). Both extracts demonstrated cytoprotective activity, with green tea showing superior efficacy, likely due to its higher catechin content. These findings suggest that green and black tea extracts, particularly green tea, have potential as natural cytoprotective agents to reduce chemotherapy-induced toxicity.

Keywords: Cytoprotective agents, Green tea, Black tea, Brine shrimp assay, Allium cepa model

1. Introduction

Cancer remains a significant global health burden, characterized by uncontrolled cell proliferation driven by genetic mutations and environmental factors. In 2020, an estimated 19.3 million new cancer cases and 10 million cancer-related deaths were reported worldwide, highlighting the critical need for effective therapeutic interventions (Sung et al., 2021). Chemotherapy, a primary treatment modality, targets rapidly dividing cancer cells but frequently causes collateral damage to healthy tissues, resulting in adverse effects such as nausea, fatigue, and organ toxicity (Hogle, 2007). To address these challenges, cytoprotective agents have been developed to protect normal cells from chemotherapeutic toxicity while preserving the anticancer efficacy of the treatment (Hogle, 2007). Among these, natural compounds, particularly polyphenols found in tea, have garnered interest for their antioxidant, anti-inflammatory, and potential cytoprotective properties (Beltz et al., 2006).

Tea, derived from Camellia sinensis, is one of the most widely consumed beverages globally and contains a rich array of bioactive compounds, including catechins in green tea and theaflavins in black tea (Li et al., 2013). Green tea is particularly high in epigallocatechin gallate (EGCG), a potent antioxidant that inhibits cancer cell survival by modulating pathways such as nuclear factor-κB and telomerase activity (Beltz et al., 2006). Black tea, despite undergoing fermentation that alters its polyphenolic composition, retains significant cytoprotective potential through theaflavins, which exhibit antioxidant and anti-inflammatory effects (Li et al., 2013). These properties position both green and black tea as promising candidates for mitigating chemotherapy-induced toxicity, particularly for drugs like methotrexate, a folate antagonist known for its cytotoxicity to both malignant and normal cells (Ujah et al., 2021).

To evaluate the cytoprotective potential of natural compounds, preclinical models such as the brine shrimp (Artemia salina) lethality assay and the Allium cepa root growth test are widely employed. The brine shrimp assay is a cost-effective and sensitive method to assess cytotoxicity and cytoprotection, using the rapid division of nauplii as a model for cellular processes similar to those in cancer cells (Zhang et al., 2012). Similarly, the Allium cepa model is a reliable tool for studying genotoxicity and cytoprotection by measuring root growth inhibition, providing insights into cellular damage and repair mechanisms (Mercado & Caleño, 2020). Both models have been validated for their ability to detect the protective effects of bioactive compounds against chemotherapeutic agents (Ali et al., 2022; Huang et al., 2002).

Despite the potential of tea polyphenols, comparative studies on the cytoprotective efficacy of green and black tea extracts against chemotherapy-induced toxicity are limited. Such studies are essential to inform dietary recommendations and develop adjunctive therapies that enhance patient outcomes during cancer treatment. This study aims to evaluate and compare the cytoprotective effectiveness of aqueous extracts of green and black tea against methotrexate-induced toxicity using in vitro brine shrimp and Allium cepa models, contributing to the evidence base for natural cytoprotective agents.

2. Materials and Methods

2.1. Materials

The present in vitro study was carried out at the Department of Pharmacy, Shree H.N. Shukla College, Rajkot, India, to evaluate and compare the cytoprotective effects of aqueous extracts of green tea and black tea using Brine Shrimp and Allium cepa (onion) models. Two commercial tea samples—Lipton Green Tea and Tata Tea Gold Care (Black Tea)—were used for extract preparation. For the Brine Shrimp model, essential materials included brine shrimp capsules (procured from Flipkart), artificial seawater (prepared using 2/3 tablespoon of sodium chloride and a pinch of sodium bicarbonate in water), standard drug solution (Gallic acid), disease control (Methotrexate), and different concentrations of the test extracts. Equipment and physical requirements included a yellow light bulb, T-valve, aerator, glass slides, petri plates, and cone-shaped bottles. For the Allium cepa model, onions, test solutions of varying concentrations, standard solutions, and a vernier caliper were utilized for the root growth measurements.

2.2. Methods

2.2.1. Preparation of Aqueous Tea Extracts

Each tea sample (10 grams) was added to 100 ml of boiling distilled water and boiled for 30 minutes. The extract was further reduced over a water bath to 10 ml to obtain the most concentrated aqueous form, representing approximately 100% extract concentration. These prepared extracts were used for subsequent biological evaluations.

2.2.2. Brine Shrimp Cytoprotective Assay

The cytoprotective activity of tea extracts was evaluated using the Brine Shrimp lethality assay. Dried cysts of Artemia (brine shrimp) were incubated in a cone-shaped bottle containing artificial seawater, continuously aerated and illuminated using a yellow light bulb to stimulate hatching. After 24 hours, the nauplii (hatched larvae) were allowed to settle by turning off the light and airflow. Ten viable nauplii were collected using a pipette and transferred into individual beakers, which were categorized into five groups: normal control, disease control, standard, and two test groups treated with different concentrations of tea extracts. Throughout the exposure period, the beakers were continuously supplied with air and light. After 30 minutes, the number of live and dead nauplii was counted. The test was continued until complete mortality was observed, and results were recorded to assess the cytoprotective efficacy of each treatment.

2.2.3. Allium Cepa Cytoprotective Assay

To further assess the cytoprotective potential of green and black tea, the Allium cepa (onion root growth) model was employed. Onions were first washed thoroughly with distilled water and then exposed to solutions containing different concentrations of the tea extracts and the standard Gallic acid solution. These onions were placed in beakers filled with respective solutions in such a way that only the basal portion remained submerged, allowing roots to grow downward into the liquid medium. The set-up was maintained for approximately one week. Throughout the period, root growth was monitored and measured regularly using a vernier caliper. The number of roots and their lengths were recorded to evaluate any growth-promoting or inhibitory effects induced by the tea extracts.

2.2.4. Statistical Analysis

The data obtained from both models were subjected to statistical analysis using one-way ANOVA to determine the significance of variance among groups. Results with $p \leqslant 0.05$ were considered statistically significant, while values of $p \leqslant 0.001$ were considered highly significant.

3. Results

3.1. Brine Shrimp Cytoprotective Assay

The Brine Shrimp lethality test was used to evaluate the cytoprotective potential of green tea and black tea aqueous extracts compared with the standard drug Gallic acid, disease control (Methotrexate), and untreated control (water). The survival of brine shrimp nauplii was recorded at 30-minute intervals over a period of 150 minutes.

At the end of 150 minutes, the control group exhibited moderate decline in cell viability with 5 out of 10 nauplii surviving, whereas complete mortality was observed in the Methotrexate group by 120 minutes. Gallic acid maintained high survival rates throughout the duration, with 9 nauplii alive at the end of the test. The black tea-treated group (T1) showed a gradual decline in survival, ending with 7 nauplii alive, while the green tea-treated group (T2) demonstrated better cytoprotective activity, maintaining 8 nauplii alive at 150 minutes.

The mean survival of nauplii across the test duration was 8 for the control, 4 for Methotrexate, 9.5 for Gallic acid, 8.33 for black tea, and 8.83 for green tea. ANOVA analysis revealed a statistically significant difference among the groups ($p = 0.001$). Further, Tukey's post hoc analysis showed that the cytoprotective effect of green tea was statistically significant when compared with Gallic acid ($p = 0.037$), while black tea showed a near-significant difference ($p = 0.057$). The comparison between green tea and black tea revealed no significant difference ($p = 0.410$), suggesting both teas have similar cytoprotective effects, with green tea being slightly more effective.

3.2. Allium cepa Cytoprotective Assay

In the Allium cepa root model, the effect of different treatments on root width and length was recorded at 24-hour intervals over a period of 96 hours. The Methotrexate-treated group demonstrated significant inhibition of root growth, maintaining both reduced width (0.62 mm) and length (5.6–5.7 mm) throughout the study period.

In contrast, the control group showed progressive increase in root width from 1.25 mm at 24 hours to 1.35 mm at 96 hours, and root length from 12 mm to 12.6 mm over the same period. Gallic acid and green tea-treated groups exhibited similar trends in both width and length growth. By 96 hours, both Gallic acid and green tea groups showed root widths of 0.99 mm and lengths of 12.8 mm and 12.6 mm respectively, indicating significant protection against cytotoxicity. Black tea also showed moderate protective effects, with root width reaching 0.92 mm and length reaching 12.1 mm at 96 hours.

These observations further support the cytoprotective potential of green tea and black tea, with green tea showing results closely comparable to the standard antioxidant, Gallic acid.

4. Discussion

The current study investigated the cytoprotective effects of aqueous green and black tea extracts against methotrexate-induced toxicity using in vitro brine shrimp (Artemia salina) and Allium cepa models. The results indicate that both tea extracts exhibit significant cytoprotective activity, with green tea demonstrating greater efficacy than black tea. These findings contribute to the growing evidence supporting the protective role of tea polyphenols in mitigating chemotherapy-induced cellular damage (Beltz et al., 2006; Hensley et al., 2009).

In the brine shrimp assay, green tea extract (T2) sustained higher nauplii survival (mean 8.83) compared to black tea extract (T1, mean 8.33) after 150 minutes of methotrexate exposure, significantly outperforming the methotrexate control (mean 4, p=0.001). This suggests that green tea's high content of epigallocatechin gallate (EGCG) may provide superior protection against oxidative stress and apoptosis induced by methotrexate (Mokra et al., 2022). Lambert and Elias (2010) reported that EGCG inhibits reactive oxygen species (ROS) generation, which aligns with the observed protection of rapidly dividing nauplii, a model for cellular proliferation akin to cancer cells (Lambert & Elias, 2010). Black tea, while effective, showed reduced efficacy, likely due to its lower catechin content following fermentation, which shifts its polyphenolic profile toward theaflavins (Yang et al., 2009). Yang et al. (2009) noted that theaflavins possess antioxidant properties but are less potent than catechins in neutralizing ROS, potentially explaining black tea's lower cytoprotective effect (Yang et al., 2009).

The Allium cepa model reinforced these findings, with green tea extract promoting greater root length (12.6 mm) and width (0.99 mm) compared to black tea (12.1 mm and 0.92 mm) after 96 hours, relative to the methotrexate control (5.7 mm and 0.62 mm). These results suggest that tea extracts counteract methotrexate's inhibition of cell division, a hallmark of its cytotoxicity (Leme & Marin-Morales, 2009). Leme and Marin-Morales (2009) established the Allium cepa assay as a sensitive tool for detecting cytotoxic and genotoxic effects, and our data indicate that tea polyphenols mitigate these effects, likely through antioxidant and DNA-protective mechanisms (Leme & Marin-Morales, 2009). Green tea's enhanced efficacy may be attributed to its catechin-rich composition, which has been shown to reduce DNA damage in plant-based models (Gupta et al., 2009).

The standard drug, gallic acid, exhibited the highest cytoprotective activity, with nauplii survival of 9.5 and Allium cepa root growth comparable to the water control. This is consistent with Kahkeshani et al. (2019), who highlighted gallic acid's potent antioxidant and free radical-scavenging properties (Kahkeshani et al., 2019). However, the Turkey post hoc analysis revealed p-values of 0.037 (green tea vs. gallic acid) and 0.057 (black tea vs. gallic acid), suggesting that green tea's cytoprotective effects are close to those of gallic acid, positioning it as a viable natural alternative. The non-significant difference between green and black tea (p=0.410) indicates shared polyphenolic mechanisms, though green tea's superior performance warrants further exploration of catechin-specific effects (Caruana & Vassallo, 2015).

The observed cytoprotective effects are likely driven by the antioxidant and anti-inflammatory properties of tea polyphenols, which counteract methotrexate-induced oxidative stress (Frei & Higdon, 2003). Frei and Higdon (2003) demonstrated that tea catechins scavenge free radicals and enhance cellular antioxidant defenses, supporting our findings (Frei & Higdon, 2003). Limitations of this study include the reliance on in vitro models, which may not fully reflect human physiological responses. Future research should incorporate mammalian cell lines or in vivo models to validate these results and investigate molecular pathways, such as the role of superoxide dismutase or Nrf2 activation (Sang et al., 2011).

5. Conclusion

This study demonstrates that aqueous extracts of green and black tea possess significant cytoprotective activity against methotrexate-induced toxicity in in vitro brine shrimp and Allium cepa models, with green tea exhibiting greater efficacy. These findings highlight the potential of tea polyphenols, particularly catechins, as natural cytoprotective agents that could complement chemotherapy by reducing its adverse effects. Further research is needed to confirm these effects in clinical settings and to optimize the therapeutic application of tea extracts in cancer management.

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