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Research article

Dose-Dependent Genoprotective Effects of *Syzygium aromaticum* (Clove) Extract Against Cyclophosphamide-Induced Chromosomal Damage and Micronucleus Formation in Swiss Albino Mice

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Abstract: Cyclophosphamide (CP), a classical alkylating chemotherapeutic drug, has significant genotoxic effect but its use is restricted due to safety concerns. In the present study, we investigated a protective effect of Syzygium aromaticum (clove) ethanolic extract against CP-induced chromosomal damage in Swiss albino mice. Thirty male mice were distributed among five groups (n = 6): control, clove extract only (500 mg/kg), CP only (20 mg/kg intraperitoneally), and two post-treatment groups, receiving CP followed by oral clove extract at 400 mg/kg and 500 mg/kg for seven days. Chromosomal anomalies and micronucleus formation were assessed in bone marrow cells. CP treatment significantly increased structural chromosomal anomalies from 2.24 ± 1.01 (control) to 18.72 ± 0.05 ($p < 0.05$) and numerical aberrations from 3.62 ± 0.03 to 15.68 ± 0.02 ($p < 0.05$). Micronucleated polychromatic erythrocytes (MnPCEs) increased 9.9-fold from 1.38 ± 0.02% to 13.63 ± 1.01% ($p<0.05$). Post-treatment with 400 mg/kg clove extract reduced structural and numerical aberrations to 10.94 ± 3.01 and 11.03 ± 0.03, respectively, representing 42% and 30% reductions in these aberrations. The 500 mg/kg dose achieved greater protection, reducing structural aberrations by 74% (4.92 ± 0.03) and numerical aberrations by 68% (5.03 ± 0.05), approaching control values. MnPCE frequency decreased to 8.84 ± 1.02% (35% reduction) and 4.98 ± 0.05% (63% reduction) at 400 mg/kg and 500 mg/kg doses, respectively. The dose-dependent genoprotective effects are attributed to the high eugenol content and phenolic compounds in cloves, which possess antioxidant properties. These findings suggest that clove extract may be a promising natural chemoprotective agent for mitigating CP-induced genotoxicity, warranting further investigation for potential clinical applications.

Keywords: *Syzygium aromaticum*; cyclophosphamide-induced genotoxicity; chromosomal aberrations; micronucleus test; chemoprotective agents

1. Introduction

Cancer remains one of the leading cause of death worldwide, which makes the need for further development and improvement of chemotherapeutic approaches essential. Alkylating agents are one of the main classes of chemotherapeutics, which is capable of treating a broad range of malignancies. Cyclophosphamide (CP) is a nitrogen mustard derivative and one of the most widely used alkylating agents in clinical oncology to-date, for treatment of

lymphomas, leukemias, breast cancer, ovarian cancer and some autoimmune diseases [1]. The clinical application of CP is heavily limited by its dose dependent toxicity, most notably the highly genotoxic effects on fast dividing normal cells. The anticancer action of CP is thought to be mediated via its metabolic activation by hepatic cytochrome P450 enzymes such as CYP2B6 and CYP3A4 with the production of phosphoramide mustard and acrolein as major metabolites [2]. Phosphoramide mustard is the active therapeutic metabolite causing DNA cross-linking and subsequent cell death in cancer cells. However, efects of this mechanism are not specific and it affects in non–selective manner even to normal cells, particularly the cells showing high rate mitosis such as bone marrow cells, intestinal epithelial and reproductive tissues. CP's toxicity is also driven in large part by acrolein, its other major metabolite, which induces the production of reactive oxygen species and kills cells directly. This results in RONSs generation (reactive oxygen and nitrogen species) which can also be scavenged by cellular antioxidant system. This will cause the cell's own antioxidant pool to be depleted, and hence results in profound DNA insults such as chromosomal aberrations, sister chromatid exchange , micronucleus formation [3].

The acute residual side effects are not the only genotoxic consequences of CP, this compound can also induce late reactive damage at cellular level which is correlative with an increased risk for secondary tumours, reproductive losses and hereditary mutations. Physical ACP is reported to increase the frequency of chromosomal aberration both in the somatic cells (cytogenesis) and germ-line chromosomes, which raises the concerns on long-term genetic security for cancer survivors [4]. These side effects require the design of strategies to protect from CP-associated genotoxicity without compromising therapeutic effect. Several natural products were proposed as potential chemoprotective agents, representing different strategies for protection against chemotherapy toxicity. Plants as Drugs: Traditional and Modern Utilization 39 applied for thousands of years in traditional medicine, may be considered as a large drug store with an unknown number of bioactive compounds having various pharmacological actions [2]. These plants are sources of a wide range of complex mixtures secondary metabolites such as phenolics, tannins, saponins, steroids flavonoids, terpenoids and alkaloids etc., which contribute to their medicinal potential [2]. The identification, description and development of antimutagens from plants during the last 40 y have radically changed our perspective concerning natural chemoprotection; many studies support an antimutagenic activity in a variety of plant extracts [2, 5]. Some plant extracts have shown protective effects against CP-induced genotoxicity in recent studies. Mice treated with Origanum vulgare extract exhibited lower chromosomal damage induced by CP and reduced oxidative stress markers [6]. As another essential oil of Foeniculum vulgare it has been revealed the marked antimutagenicity, which is attributed the antioxidant content [7]. *Crataegus microphylla (hawthorn) extract too had a protective role, reduced structural and numerical AAs in CP rats* [8]. Taken together, these observations indicate that dietary plant antioxidants have the potential to be effective at mitigating oxidant stress-induced genetic damage by multiple modes of action, including free radical scavenging activity and stimulation of cellular antioxidant systems as well as interactions with metabolizing enzymes. Syzygium aromaticum (Myrtaceae) is one of the most valuable medicinal spices and has been commercially used in diverse therapeutic domains. Cubeb originated in the Maluku Islands of Indonesia and the flower buds it produces are quite aromatic and have high medicative value that's pretty unique [9]. The phytochemical of clove is remarkable that eugenol comprises between 60-90% of its EO. Other bioactive compounds are eugenyl acetate, β-caryophyllene, gallic acid and several flavonoids (kaempferol, quercetin, rhamnetin) and hydrolyzable tannins [10]. Such a variety of chemical elements lay the groundwork for the multidirectional pharmacological properties of cloves, including important antimicrobial, antidiabetic, antioxidant, anti-inflammatory and antimutagenic effects.

The radical-scavenging ability of cloves is among the highest of natural spices because its phenolic components can scavenge free radicals and chelate metal ions [11]. Eugenol, the major bioactive compound, has excellent scavenging activity for lipid peroxyl radicals, hydroxyl radicals, and superoxide anion radicals. In terms of mechanism, the antioxidant activity of eugenol is based on the donation of a hydrogen atom from the phenolic hydroxyl group to free radicals, thereby forming stable phenoxyl radicals that halt free radical-mediated chain reactions [12]. In addition, eugenol induces the expression of endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, which augment cellular defense

lines against oxidative stress. The safety and antimutagenic potential of clove extracts have already been reported by earlier workers. A standardized garlic extract (Clovinol) did not show genotoxic effects in mice at high doses and exerted significant antimutagenic activity in bacterial mutation tests [10]. The extract effectively suppressed mutations induced by various chemical mutagens, suggesting broad-spectrum protective effects [10-11]. Moreover, clove oil has been shown to exhibit cytoprotective effects against hydrogen peroxide-induced oxidative damage in cellular systems as well [10]. There is a need for sensitive and dependable biomarkers in the evaluation of genotoxicity and chemoprotective efficacy. Cytogenetic analysis of chromosomal anomalies in bone marrow metaphase cells is the gold standard for assessing clastogenic effects, where structural and numerical damage to chromosomes can be directly observed [13]. In the micronucleus test, a helpful addition to the analysis of chromosomal aberrations, chromosome segments or even entire chromosomes that are not integrated into the main nuclei during cell division are counted [14]. These cytogenetic endpoints are of particular interest in the evaluation of CP-induced damage, because it has been repeatedly reported that CP causes an increase in chromosomal aberrations and micronucleus formation [3].

Since the genotoxic and chromosomal effects of CP have been well documented in the literature, and a promising protective effect has been recorded by natural antioxidants, these studies aimed to investigate the chemoprotective nature of *Syzygium aromaticum* ethanol extract on CP-induced chromosomal damage in Swiss albino mice. The specific aims are to investigate the dose-dependent effects of clove extract on structural and numerical chromosomal anomalies and its interactions with clastogenic damage, as well as to form micronuclei in polychromatic erythrocytes, and to determine the most effective protective dose. This study contributes to the growing evidence suggesting that natural agents can serve as adjuvants in cancer treatment, potentially enhancing the therapeutic index of standard chemotherapy.

2. Materials and Methods

2.1 Chemicals and Reagents

Cyclophosphamide (CP) was obtained as a pharmaceutical-grade powder (Endoxan, Baxter Oncology GmbH, Germany). Colchicine, potassium chloride (KCl), and Giemsa stain were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS), absolute methanol, and ethanol were of analytical grade and procured from local suppliers. All chemicals and solvents, unless otherwise stated, were of the highest commercial purity.

2.2 Plant Material and Extract Preparation

The flower buds of clove (*Syzygium aromaticum*) were procured from local markets in Al-Qadisiyah Province, Iraq. For correct authentication, the sample was confirmed by experts in the Department of Biology, College of Education, University of Al-Qadisiyah, and a voucher specimen (collector A.A.-RK-52) was maintained in the herbarium. Ground dried buds were obtained by milling in an electric grinder to a fine powder and standardized by sifting through a 40-mesh sieve. The extraction procedure of Tajuddin et al. [15] was adapted. In this process, 100 g of clove powder was subjected to Soxhlet extraction using 300 mL of 50% ethanol (v/v) at a temperature range of 60–70°C for six hours. The solution was then screened through Whatman No. 1 paper, and the solvent was concentrated at 40 °C under diminished pressure using a rotary evaporator (Buchi Rotavapor R-205). The by-product is a semi-solid extract of a yellow color and a characteristic odor. The extract was weighed and the yield noted; it was then stored in amber-colored glass bottles at 4°C until further use. Dilutions were freshly made in distilled water to the desired concentrations for animal treatment.

2.3 Experimental Animals

Swiss albino male mice (Mus musculus), 6–7 weeks of age and weighing 25–30 g, were obtained from the animal house of the College of Education, University of Al-Qadisiyah. The mice were given a one-week adaptation period under standard environmental conditions (temperature of 22 ± 2°C, relative humidity of 55 ± 10%, and a light/dark photoperiod of 12 h) prior to the initiation of the experiment. The mice were housed six per polypropylene cage and had wood shavings for bedding (changed twice a week). They were fed a standard pelleted diet and provided ad libitum access to tap water throughout the study. All experiments

were conducted in accordance with the guidelines of the local ethics committee for experimental animal studies, following approval.

2.4 Dose Selection and Preparation

The CP dose (20 mg/kg body weight) was selected from previous genotoxicity studies as a dose that induces persistent chromosomal aberrations without excessive mortality [4]. The CP solution was prepared immediately before application by dispersing 2 mg of cyclophosphamide powder in 10 mL of deionized water to the required concentration for intraperitoneal injection. The clove extract doses of 400 mg and 500 mg per kg body weight were determined after initial dose-finding experiments and based on previous investigations regarding the safety range of clove extracts [10]. The extract solutions were freshly prepared daily by dissolving the extract in distilled water to obtain a concentration that allowed for an administration volume of 0.2–0.3 mL per mouse.

2.5 Experimental Design

Thirty mice were randomly allocated among five groups (n = 6 per group) using a random number table. Group 1 (Control): Administered distilled water (0.3 mL) orally for seven days. Group 2 (Clove): They received a clove solution orally at a dose of 500 mg/kg body weight for a period of seven days. Group 3 (CP only): Treated with a single intraperitoneal treatment with CP (20 mg/kg body weight) on day 1 and distilled water orally for seven days. Group 4 (CP + Clove 400): Administered a single intraperitoneal injection of CP (20 mg/kg) on day 1 and treated orally with clove extract (400 mg/kg body weight) for 7 days. Group 5 (CP + Clove 500): CP was injected intraperitoneally in a single dose (20 mg/kg) on day 1, followed by the dispensation of clove extract at a concentration of 500 mg per kg body weight for seven days orally. All oral gavages were conducted using a feeding needle, and the treatments were administered daily at equal time intervals (9:00 AM) in order to minimize circadian rhythm fluctuations.

2.6 Chromosomal Aberration Assay

The chromosomal anomaly assay was performed using the method of Allen et al. [13], with minor modifications. On day 8 of treatment, animals were injected intraperitoneally with colchicine (0.25 mL, 10 mg/kg body weight) to arrest dividing cells at metaphase 90 minutes before sacrifice. Mice were humanely killed by cervical dislocation: femurs were excised and stripped of soft tissue. Bone marrow was washed out of each femur with 5 mL phosphate-buffered saline (PBS) and transferred into centrifuge tubes using a 25-gauge needle. The suspension was subjected to centrifugation for 10 minutes at 2000 rpm at ambient temperature, and the liquid phase was removed. The pellet was gently resuspended in 5 mL of preheated hypotonic potassium chloride solution (0.075 M, 37°C) and incubated in a water bath for 20 minutes with intermittent mixing every 5 minutes. The cells were spun down at 2000 rpm for 10 minutes again after hypotonic treatment. The supernatant was removed, and the fixation process was initiated by the slow, dropwise addition of 5 mL of fresh Carnoy's fixative (methanol: acetic acid, 3:1) with gentle shaking. The fixation procedure was performed three times. The last pellet was resuspended in 500 µL of fixative, and ~approximately 4–5 drops of the liquid phase were carefully dribbled down onto pre-cleaned, cooled glass slides from a height of ~approximately 30 cm. The slides were placed on a hot plate at 50 °C and stained with 5% Giemsa for 10–15 min. A total of 100 metaphase plates per animal were scored for analysis under oil immersion at 1000× magnification. Both structural changes (chromosome breaks, chromatid breaks, fragments, rings, and dicentrics) and numerical changes (aneuploidy and polyploidy) were scored, and the percentage of abnormal cells was calculated.

2.7 Micronucleus Test

Micronucleus assay: The micronucleus assay was conducted using the method described by Schmid [14]. Bone marrow cells were prepared by flushing the femur with 1 mL of heat-inactivated fetal bovine serum. The suspension prepared was then spun down at 1000 rpm for 5 minutes. 1 drop of this concentrated sedimented cell pellet was spread over a clean glass slide to make a smear, which was allowed to air-dry and further processed accordingly. The slides were then air-dried for 24 hours at room temperature, fixed in absolute methanol for 5 minutes, and dyed with May-Grünwald-Giemsa. For scoring, 1000 polychromatic

erythrocytes (PCEs) were evaluated per animal in oil immersion at a magnification of ×1000 to detect micronuclei. The micronucleus index was expressed as the number of micronucleated cells per 1000 cells by the following formula:

Micronucleus Index (%) = (Number of micronucleated PCEs / Total number of PCEs examined) × 100

Bone marrow toxicity was also estimated by determining the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) based on 500 counted cells.

2.8. Statistical Analysis

Data are presented as mean ± SE. Data were analyzed with SPSS (version 25.0). One-way ANOVA was used for comparison among groups, and then Tukey's post hoc test was performed to compare any two groups. The cut-off point for statistical significance was $p < 0.05$.

3. Results and Discussion

3.1 Effects of Clove Extract on Cyclophosphamide-Induced Chromosomal Aberrations

Analysis of bone marrow metaphase cells revealed that cyclophosphamide (CP) treatment induced significant chromosomal damage in Swiss albino mice (Table 1). The structural chromosomal aberrations in the CP-only group (18.72±0.05) were 8.3-fold higher than the control group (2.24±1.01, $p<0.05$). Likewise, the rate of numerical abnormalities was 4.3 times higher in CP-treated versus control animals (from 3.62±0.03 to 15.68±0.02; $p<0.05$). The clove extract alone group did not significantly differ from the control, and had number of structural aberrations of 3.02±0.62, and that of numerical aberrations 3.39±1.01, indicating non-genotoxic potential of the extract at tested concentration (500 mg/kg b.wt). The forms of observed structural aberrations were chromatid breaks (42%), chromosome fractures (28%), gaps (15%), ring chromosomes (10%) and dicentric chromosomes (5%). This pattern of anomalies is consistent with CPs alkylating mechanism that gives rise to DNA interstrand cross-links and subsequently leads to chromosome breakage at S-phase [1]. The preferential formation of chromatid-type aberrations indicates the induction of damage in late S phase, indicative of CP's cell cycle-specific genotoxicity [3]. Post-treated with clove ext proved to be quite remarkable and dose-dependent protection. At a dosage of 400 mg/kg, structural chromosomal aberrations decreased to 10.94±3.01 (41.5% reduction) in contrast with the CP-only group and numerical ones were 11.03 ± 0.03 (29.6% reduction). Most importantly, the 500 mg/kg dose provided near-complete protection with a reduction of structural and numerical aberrations to 4.92±0.03 (73.7% reduction) and 5.03±0.05 (67.9% reduction), respectively, which were not significantly different from control levels (P>.05). These results are in agreement with antecedent studies that proved the protective activity of phenolic-rich plant extracts against CP-induced genotoxicity. Shokrzadeh et al. observed that extract of Origanum vulgare decreased CP-induced chromosomal aberrations in mouse lymphocytes by 60-70%, due to its high level of rosmarinic acid and carvacrol [6]. Similarly, Yonekubo et al. This last work reported a 55% reduction in chromosomal aberrations by using Crataegus oxyacantha extract, although with higher doses compared to the present study [8].

Table 1. Impact of clove extract on cyclophosphamide-induced chromosomal anomalies in bone marrow cells of Swiss albino mice

Treatment Groups	Structural Chromosomal Aberrations	Numerical Chromosomal Aberrations	Total Aberrant Cells	Protection (%)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Control	2.24 ± 1.01^{a}	3.62 ± 0.03^{a}	5.86 ± 1.04^{a}	-
Clove only (500 mg/kg)	3.02 ± 0.62^{a}	3.39 ± 1.01^{a}	6.41 ± 1.63^{a}	-
CP only (20 mg/kg)	18.72 ± 0.05^{b}	15.68 ± 0.02^{b}	34.40 ± 0.07^{b}	0
CP + Clove (400 mg/kg)	10.94 ± 3.01^{c}	11.03 ± 0.03^{c}	21.97 ± 3.04^{c}	42.5
CP + Clove (500 mg/kg)	4.92 ± 0.03^{a}	5.03 ± 0.05^{a}	9.95 ± 0.08^{a}	82.7

Values are the means ± SE of 100 metaphase cells per animal (n=6 for each group). Values with different superscripts within the same column are significantly different ($p < 0.05$, one-way ANOVA followed by Tukey's test). Protection (%) = [(CP only - Treatment group) / (CP only - Control)] × 100.

3.2 Micronucleus Formation and Cytotoxicity Assessment

The micronucleus tests supported the chromosomal aberrations, and showed intense genotoxic stress which was triggered by CP (Table 2). MnPCEs increased dramatically from 1.38±0.02% of control to 13.63±1.01% of the CP-alone group by 9.9-fold ($p<0.05$). This large effect is consistent with massive chromosome breakage and spindle malfunction, charcterictics of the clastogenic and aneugenic effects of CP [14]. Treatment with clove extract at 400 mg/kg post-irradiation decreased the frequency of MnPCE to 8.84±1.02%, which rendered 35.1% protection. The level of PB protection at the 500 mg/kg DA-9701 dose was greater (MnPCE frequency, 4.98±0.05%, with a -63.5% reduction in CP-induced damage). Concentration-dependent accumulation of protective phytochemicals in target tissues is indicated by the dose-response curve observed ($r^2 = 0.92$). Bone marrow cytotoxicity as assessed by the PCE/NCE ratio reduced significantly in CP-only group from the control value 1.2±0.08 to 0.45±0.06, indicating strong myelosuppression (in Figure-3C). Clove extract treatment significantly protected the CP-mediated cog ratio changes and, 400 mg/kg (0.72 ± 0.05) and followed by 500 mg/kg of extracts (0.89 ± 0.07). This cytoprotective activity is of particular interest in a clinical setting, where myelosuppression is a dose-limiting toxicity of CP treatment [1].

Table 2. Impact of clove extract on cyclophosphamide-induced micronucleus formation and bone marrow cytotoxicity

Treatment Groups	MnPCE/1000 PCE	MN Index (%)	PCE/NCE Ratio	Cytotoxicity Reduction (%)	MN Protection (%)
	Mean ± SEM	Mean ± SEM	Mean ± SEM		
Control	13.8 ± 0.2[a]	1.38 ± 0.02[a]	1.20 ± 0.08[a]	-	-
Clove only (500 mg/kg)	16.1 ± 1.1[a]	1.61 ± 0.11[a]	1.18 ± 0.09[a]	-	-
CP only (20 mg/kg)	136.3 ± 10.1[b]	13.63 ± 1.01[b]	0.45 ± 0.06[b]	0	0
CP + Clove (400 mg/kg)	88.4 ± 10.2[c]	8.84 ± 1.02[c]	0.72 ± 0.05[c]	36.0	35.1
CP + Clove (500 mg/kg)	49.8 ± 0.5[d]	4.98 ± 0.05[d]	0.89 ± 0.07[d]	58.7	63.5

MnPCE, micronucleated polychromatic erythrocytes; PCE, Polychromatic erythrocytes; NCE, Normochromatic erythrocytes Data are expressed as mean value ± S.E.M Significant differences Vs each group presented by different letters ($p< 0.05$ one-way ANOVA followed by Tukey's test) MN Protection (%) = [(CP only - Treatment) / (CP only-Control)] ×100 Cytotoxicity Reduction (%) = [(Treatment - CP only)/ (Control- CP only)] ×100

3.3 Clinical Implications and Translational Potential

The dose-response curve of the observed genoprotective effect is relevant for integrative oncology. The oral bioavailability of eugenol (around 60% in humans) and its promising pharmacokinetic properties could enable clinical application [16]. In addition, the doses studied here (400-500 mg/kg in mice) would be equivalent to 2-2.5 g/day for a 60 kg human when adjusted by body surface area and are in line with traditional medicinal use [15]. Nevertheless, it should be noted for clinical application that clove extract might interact with CP in terms of its anticancer effect. Although our work has shown that normal bone marrow cells are protected, the impact on tumor cells is undetermined. "Selective cytoprotection," in which only normal cells are protected, thereby sparing them without protection of chemotherapy-induced toxicity to the tumor cell, would be a desirable situation. Similar selectivity for cancer has been observed with other non-protien antioxidants as well, possible consequence of the different redox status between normal and cancerous cells [17]. Clove extract has a favourable safety record described by comprehensive toxicological tests, which provides an anticipation for clinical application. Vijayasteltar et al. did not exhibit any toxic effects at doses as high as 2000 mg/kg in subchronic toxicity tests, nor teratogenicity against disease models of animals [10]. Furthermore, clove is recognized by the FDA as GRAS (Generally Recognized As Safe) for food applications, which in turn could speed regulatory acceptance of therapeutic use.

3.4 Study Limitations and Future Directions

Several limitations should be acknowledged. First, the study only looked at short-term acute genotoxic endpoints leaving aside late effects or delayed outcomes. Second, the precise molecular pathways responsible for protection were not directly addressed which can restrict mechanistic insight. Thirdly, it was not investigated whether clove extract compromises therapeutic effects of CP against cancer cells, which is one of the most important issues for clinical translation. These limitations have been to be addressed by future studies with mechanistic assays involving the oxidative stress markers (8-oxo-dG, lipid peroxidation products), the DNA repair enzyme activities (OGG1, XRCC1) and the apoptotic signaling pathways. The study of the influence of clove extract on CP pharmacokinetics and metabolism could help to explain drug interactions. Furthermore, in vivo tumor studies are critical to establish whether genoprotection can be achieved without adverse effects on anticancer efficacy. Standards of clove extract with a known eugenol content and potential bioavailability enhancement such as nano formulation and liposomal approach should be developed to enhance the efficacy of treatment. Treatment trials using clove extract in conjunction with chemotherapy, with concomitant measurement of toxic effects of treatment and evidence for efficacy, are the obvious next step towards clinical deployment.

4. Conclusions

The present study is the first to report significant dose dependent protection by Syzygium aromaticum ethanolic extract against cyclophosphamide-induced genotoxicity in Swiss albino mice. 500 mg/kg clove extract -post treatment demonstrated conspicuously genoprotective effect against the CTC toxicity, and significantly ameliorate the frequency of structural chromosomal aberrations (73.7%), numerical variations (67.9%) as well as micronuclei formation (63.5%) near to that of control ones. The moderate and significant protection by 400 mg/kg dose reaffirmed that Rule. This protective effect against oxidative stress may be due to the high phytochemical content in clove extract, specifically eugenol and other phenolic compounds through multiple protective mechanisms, like direct scavenging of ROS, the improvement of antioxidant endogenous systems, modulation of xenobiotic metabolism and suppression of inflammatory pathways. As clove extract is found to be non-genotoxic at therapeutic doses, and protective effect being strong, its application as a safe adjuvant therapy for cancer chemotherapy is justified. These results have important clinical implications and indicate that supplementation of clove extract may offer the opportunity to decrease the dose limiting genotoxic adverse effects produced by cyclophosphamide without causing additional toxicity. Further, the oral approach of delivery along with long history of use as a spice in different countries, emphasizes safety in gastrointestinal tract and potential clinical utility. Nevertheless, underlying inquiries on the impact on CP's anticancer effect and followed human treatment scheme remain open.

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