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Anticlastogenic activity of ethanolic extract of *Lagenaria siceraria* fruit (ELSF) against cyclophosphamide induced genotoxicity in mice

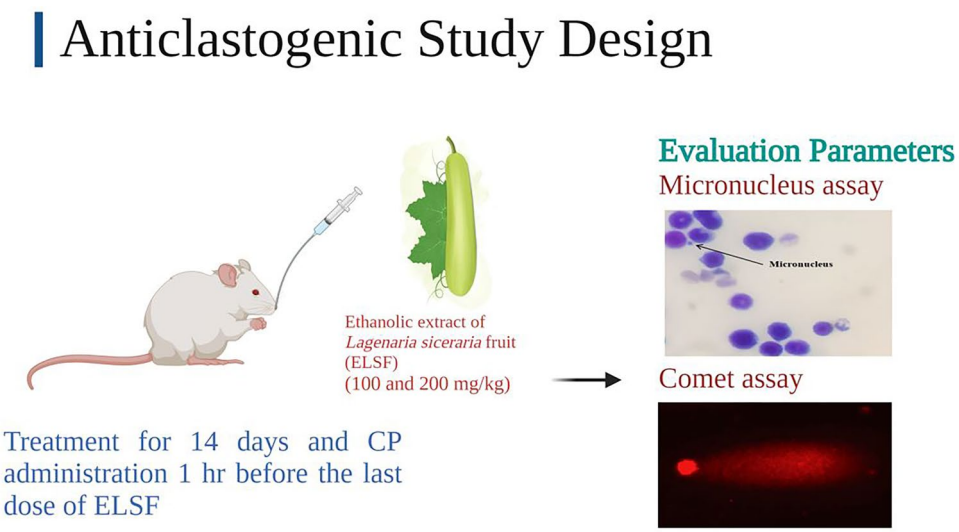
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Abstract
To investigate the potential of ethanolic extracts of *Lagenaria siceraria* fruit (ELSF) in protecting against cyclophosphamide (CP)—induced genotoxicity in Swiss albino mice. The study used a pre-treatment approach with ELSF given orally to the animals at two different doses (100 and 200 mg/kg) for 14 days straight. The CP induction group was given prior treatment for 14 days with ELSF (100 and 200 mg/kg) and the positive control group received an i.p (single intraperitoneal) cyclophosphamide dose (40 mg/kg) as the induction agent. The frequency of CP-induced micronuclei and damage to DNA was assessed as hallmark in bone marrow cells isolated form mouse. Study findings revealed that ELSF pre-treatment significantly lowered the frequency of CP-induced micronuclei and DNA damage in mouse bone marrow cells. The suppression effect as protectant was observed at both doses of ELSF (100 and 200 mg/kg). The study demonstrated that ELSF has potential chemoprotective properties against CP-induced genotoxicity. The findings suggest that ELSF could be a natural and safe approach to protecting healthy cells from the harmful effects of chemotherapy. Further clinical investigation warrants the ameliorative potential of ELSF in cancer treatment.

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Graphical abstract



Article Highlights

- *Lagenaria siceraria* is evaluated for genotoxicity potential against cyclophosphamide.
- Underlying the molecular mechanisms was screened.
- Results conclude safety and efficacy of *Lagenaria siceraria* fruit.
- Comet and micronuclei assay of ethanolic extracts of *Lagenaria siceraria* fruit.

Keywords Cyclophosphamide · Micronucleus · Anticlastogen · Comet assay · *Lagenaria siceraria*

1 Introduction

Environmental and dietary factors contribute to cancer's progression. Mutagenic and carcinogenic substances, exogenous or endogenous, can harm human DNA anytime [1]. Endogenous factors play a role in transmitting the potential biological effects, in contrast to the majority of exogenous factors like physical and chemical carcinogens. Hydroxyl radicals, superoxide radicals, reactive oxygen radicals (ROS), peroxide radicals, and oxyions are endogenous factors. They are reactive oxygen-containing chemical compounds generated as metabolic waste [1].

Cyclophosphamide (CP) has demonstrated beneficial target in treating diverse group of cancer including Hodgkin's disease, multiple myeloma, ovarian cancer, lung cancer, and breast cancer, when paired with other drugs [2]. CP is commonly utilized as an immunosuppressive medication for curative purpose in autoimmune disease types like rheumatoid arthritis (RA) and systemic lupus erythematosus. It is frequently administered alongside other immunosuppressants such azathioprine, mycophenolic acid, or prednisone [3, 4]. The potential negative consequences of CP treatment encompass the inhibition of bone marrow cells, nephrotoxicity, and heightened vulnerability to infection, infertility, cardiovascular complications, and the emergence of secondary malignancies [5].

DNA arrays such as inter/intra crosslinks, protein cross-links and, monoadducts of DNA are the cause of CP's cytotoxic effects. The occurrence of DNA strand breaks is closely associated with the production of DNA adducts [6]. Upon encountering DNA damage, a series of interconnected processes are triggered, including repair mechanisms to fix the damage and mechanisms to accelerate the apoptosis process [7]. The cell signaling pathways that are mediating apoptosis must have a significant influence over the cell modulation response to CP. CP creates micronuclei, which can result in cell death, genetic instability, or the formation of cancer [8]. CP exposure leads to an increase in the occurrence of mutant red blood cells, which subsequently harms blood-forming cells and elevates the likelihood of developing secondary leukemia.

Combining CP with medication is a common practice that has been shown to be beneficial [9]. Medication combinations are now being investigated to enhance the efficacy of destroying cancer cells while minimizing the adverse effects on healthy cells. The micronucleus screening test is used to assess genotoxicity. The alkaline comet assay is a practical extension of the micronuclei (MN) test [10].

Micronuclei (MN) are generated during cell division when a small acentric chromatin fragment is not entirely segregated during anaphase and becomes visible as a micronucleus during interphase if a chromosome or chromosomal fragment is not separated [11]. The MN test was developed to expedite quick screening. Heddle [12], while studying bone marrow erythrocytes, chose to utilize the term "micronucleus," which had already been employed by Boller and Schmid [13] to characterize the genotoxic discoveries. The micronucleus test has gained popularity as a screening technique owing to its user-friendly nature, long-lasting performance, high sensitivity, and capacity to accurately identify strand breaks at the genetic level [14].

The gel electrophoresis (single cell-alkaline comet method) is a straightforward method targeting alkaline label sites proven effective for assessing genomic instability, alkali reactivity sites, and repair in individual prokaryotic and eukaryotic cells. This assay is valuable in various fields, including hereditary toxicity and biological epidemiological investigations [1]. Measuring DNA damage and repair in humans and conducting basic genotoxicity assays has become a common practice. Assays employing micronucleus and comet DNA fragmentation techniques are useful for assessing genotoxicity in animal models [15].

Several built-in entities and mechanisms protect DNA from radiation and chemicals to maintain genetic information and its intended function. Natural antioxidants modify intracellular redox potential; suppress ROS synthesis, and scavenging other free oxygen radicals [16, 17]. Vitamins A, C, and E, carotenoids, polyphenolic substances, and flavonoids shield cells from free radicals impact and deduct the risk of chronic disease. Due to their high efficacy against mutation and carcinogenesis, phytochemicals, flavonoids, and plant-based diets are prominent chemopreventive agents [18].

The well-known vegetable plant *Lagenaria siceraria* (Molina) belongs to the Cucurbitaceae family and is locally popular as Ghiya, Lauki, and Bottle Guard [18]. Due to the presence of all the necessary components for quality and health, this plant's fruit, leaf, and seeds provide excellent health effects. Lagenin, a key ingredient in these seeds and fruit, has been shown to have excellent anticancer [19], anti-hyperlipidemic [20], immunosuppressive [19], hepatoprotective, antiproliferative [18], antiviral, and anti-HIV effects [21].

To combat CP's clastogenic effect on Swiss albino mouse bone marrow cells, we aimed to test the ELSF's anticlastogenic and antigenotoxic properties against CP using micronucleus and comet assay tests for the present investigation.

2 Materials and methods

2.1 Ethanolic extract of *Lagenaria* siceraria fruit (ELSF) preparation

Fruits of *Lagenaria siceraria* (Molina) Perennial climber collected from local market of Mysore and samples were Identified and authenticated as per the protocol mentioned in Ayurvedic Pharmacopoeia India (Part I Volume 3 pp. 216–217) by the Pharmacognosy department, JSS College of Pharmacy, Mysuru (Voucher no: 1311). Then Fruits ware peeled, and then shade-dried until they reached a constant weight. The powder was made by pulverising the dried pieces in an electric blender. For four days at 50–60 °C, 100 g of dried, coarsely ground fruit was extracted using 90% ethanol in a soxhlet apparatus [22, 23]. The mixtures were filtered afterward. Finally filtrate at 45 °C was concentrated on a rota evaporator to remove the traces of ethanol; the extracts were placed in sterile bottles and stored in the refrigerator until use. Followed by sample has been deposited in depository of department of Pharmacology for future reference (JSS/Pharmacol/PhD/PS: 02/02-09-2022).

2.2 Chemicals and reagents

All chemicals, including cyclophosphamide, were purchased from Sigma Aldrich and Himedia.

2.3 Experimental animals

The research was conducted using Swiss albino mice, which were housed in the institution's animal facility (Centre for Experimental Pharmacology and Toxicology, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India).

Mice were housed in well-conditioned room for 24 h period at 27 ± 2 °C with a RH 40–50%, in cages resembling polypropylene shoe boxes and feeder with pellets (Amruth Feeds, India) with free access to water. Tests were conducted on animals aged 8–10 weeks old and weighed on an average of 25 ± 2 g. A total of 36 Swiss albino mice were segregated randomly into six groups. Every group had access to a total of six animals (three males and three females) housed in separate enclosures [24, 25]. The groups selected in this study: a control group, two ELSF groups, a CP group, and two groups receiving ELSF + CP at varying doses. Animal care and experimentation followed protocols approved by the CCSEA. Following the recommended procedures and adhering to the highest ethical standards, we provided the best possible care for all animal species. The Institutional Animal Ethics Committee (IAEC) of JSSCP in Mysuru, Karnataka, India, reviewed and approved all experimental protocols (IAEC/JSSCPM/341/2019) before any animal testing was performed.

2.4 Dose and treatment schedule

According to prior research [26, 27], two separate doses of ELSF (100 and 200 mg/kg, b.w) included for our study. Control group treated with normal saline (p.o), positive control group treated with cyclophosphamide (CP-40 mg/kg/bw, i.p) single dose, treatment group pre-treated with ELSF (100 and 200 mg/kg/bw, p.o) for 14 days and CP administrated 1 h before on the last dose of ELSF [7, 28, 29]. Cells from bone marrow of mice were incised and modified for comet and micronucleus assay for further processing post the treatment for 24 h.

2.5 Bone marrow micronucleus assay

The bone marrow micronucleus (BMN) assay was accordance with Schmid method [30], with minor modifications made by Seetharam et al. [31] in accordance with OECD guideline (474) [32]. After 2 mL of three percent bovine serum albumin (BSA) was added to the BM cells from the tibia and femur bones, the resultant centrifuge of cell suspension at 1500 rpm for 10 min. To obtain a solution of the proper thickness, the pellet was again suspended in the necessary amount of 3% BSA after the supernatant was removed. A suspension drop was applied to a spotless slide, allowed to air dry, and then fixed for ten minutes in methanol. Giemsa stain (10 min) and May-Grunwald's (15 min) were applied to fixed slides. After rinsing the slides twice or three times in phosphate buffer, they were placed in distilled water for a minute to allow the immature and adult erythrocytes to properly differentiate. Under an Olympus BX51 microscope (600dpi image, 100 × maginification), screened for slides to ensure the micronuclei presence in poly and normochromatic erythrocytes (PCE and NCE). PCE staining is blue, while NCE staining is reddish pink. Further based on this data the PCE/NCE ratio was also calculated. A total of 2000 PCE and matching NCE observed over the field were subjected for MN presence from each animal.

2.6 Comet assay

The comet assay samples were prepared following the protocol established by Singh et al. [33] and OECD guideline (489) [34]. Slides were stained by dropping 60 µl of a 20 µg/ml ethidium bromide (EtBr) solution dropwise for two minutes prior to image processing. EtBr-stain was used to visualize the damage to DNA. One hundred cells from each animal were examined using a fluorescent microscope with a 40 × objective (EVOS-FL cell imaging system). The staining process reveals the extent of the tail formation damage, which is reflected in the grades. These comets were sorted into five groups, or degrees of damage, based on the amount of DNA found in their tails, and then visually scored and analysed. No harm was done; the score was 0. Damage levels from 1 to 4 indicate mild, moderate, high, and severe levels of disruption. After being stained, the damage extent caused by formation of tail can be seen, and this is reflected in the grades.

A CCD camera was utilized to take pictures of fifty randomly chosen cells. The images were then processed with Open Comet (Open Comet v1.3.1) software, which allows for automatic analysis of comet test images. For every sample, the percentage of Tail DNA was recorded. The olive moment and tail moment were subsequently determined to be the optimal parameters among the others.

$$\text{Olive moment} = (\text{tail mean} - \text{head mean}) \times \% \text{ tail DNA} \tag{1}$$

$$\text{Tail moment} = \frac{\text{tail length} \times \% \text{ tail DNA}}{100} \tag{2}$$

2.7 Statistical evaluation

One way ANOVA and Dunnett's test post hoc performed in Graph Pad Prism 9 determined the statistical significance (Graph Pad Software, Inc., CA, USA). Comparisons reflected between the ELSF treatment groups and a control group. Groups receiving CP treatment were compared to those receiving ELSF + CP. P-value ≤ 0.05 indicate statistical significance or indifferences. Results were considered significant if they were labelled as $*^{\#}P < 0.01$, and $**^{\#\#}P < 0.001$.

3 Results

3.1 Effect of ELSF on Cyclophosphamide induced MN in bone marrow (BM) cells of mice

The positive agent CP group has shown prominent increase in the PCE pertaining MN quantity in bone marrow cells of mice as shown in Fig. 1. There was dose dependent increase in MN with CP induction. At the same time CP also showed the decline in PCE/NCE ratio. The test group of ELSF 100 and 200 mg/kg have shown reduction in MN frequency comparable to the induced group. The two test doses of ELSF were proven beneficial against CP and the lowest dose 100 mg/kg was more efficient than the higher. The significance rise and reduction effect of CP comparable to ELSF doses were presented in Fig. 2 and Table 1.

3.2 Effect of ELSF on cyclophosphamide induced DNA damage bone marrow (BM) cells of mice

The positive agent CP has shown marked rise in the comet number from 1 to 4 comparable to the control group. The ability to cause DNA damage by the CP is accountable for its genotoxicity. The test groups induced with 100

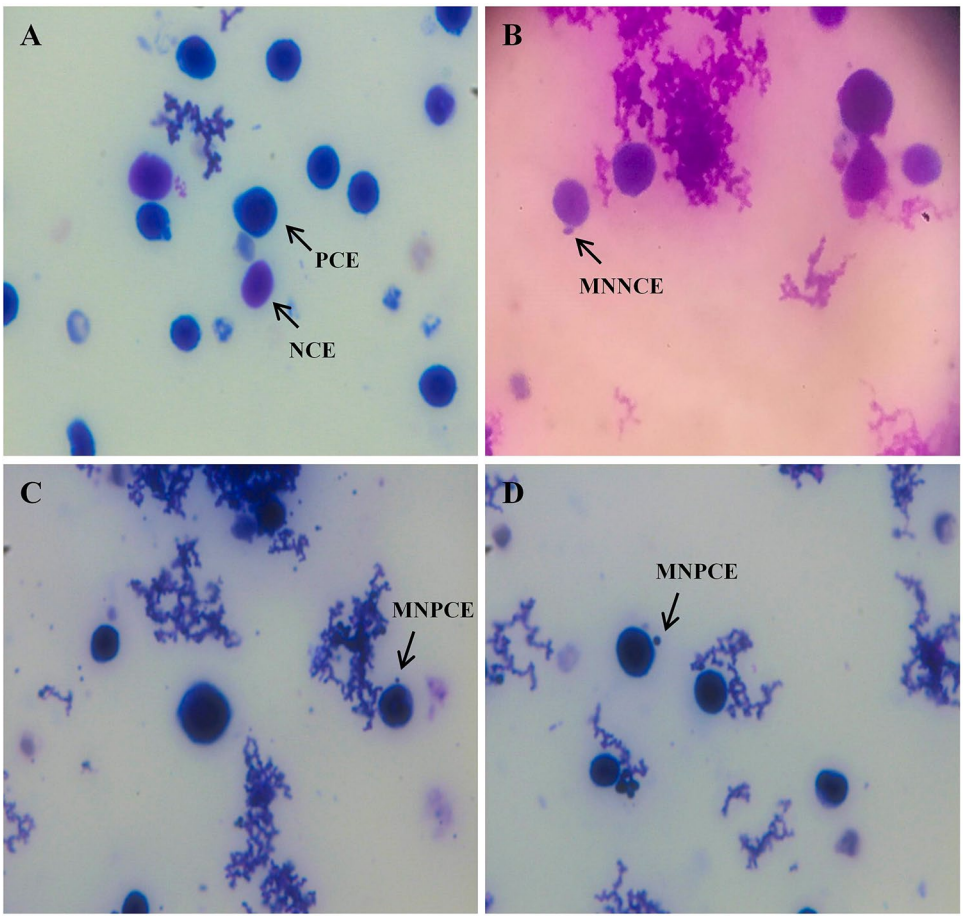


Fig. 1 Microscopic evidence of micronucleus formation in mouse bone marrow cells after cyclophosphamide treatment. **A** Normochromatic erythrocytes (NCE); polychromatic erythrocytes (PCE). **B** Normochromatic erythrocytes with the micronucleus (MNNCE). **C** and **D** Polychromatic erythrocytes with the micronucleus (MNPCE). Significant decrease in number of micronuclei in the treatment group was observed

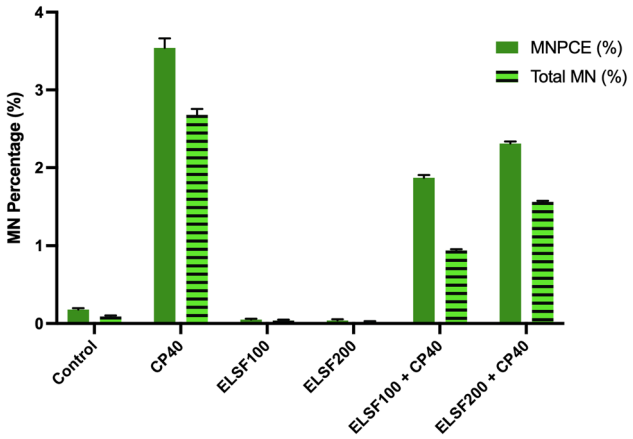


Fig. 2 Frequency of MN in Cyclophosphamide- and ELSF-treated mice's bone marrow cells, percentage of MNPCE (Micronucleus in polychromatic erythrocytes) and total MN (micronucleus in polychromatic erythrocytes and normochromatic erythrocytes) was evaluated in each mouse. Mean ± Standard Error (n = 6), Analysis of variance (ANOVA) with a post hoc Dunnett's test. Significant at the **P < 0.001 level when compared to the control group, ##P < 0.001 when compared to cyclophosphamide. Significant decrease in number of micronuclei in the treatment group was observed

Table 1 Frequency of MN in cyclophosphamide- and ELSF-treated mice's bone marrow cells

Treatment (mg/kg)	MNPCE (%)	Total MN (%)	PCE/NCE
Control (0.9% saline)	0.18 ± 0.016	0.09 ± 0.014	1.23 ± 0.025
Positive control (CP40)	3.54 ± 0.124**	2.68 ± 0.075**	0.74 ± 0.021**
ELSF100	0.05 ± 0.012**	0.04 ± 0.011**	1.42 ± 0.004**
ELSF200	0.04 ± 0.014**	0.02 ± 0.010**	1.38 ± 0.008**
ELSF100 + CP40	1.87 ± 0.035##	0.94 ± 0.015##	0.82 ± 0.005##
ELSF200 + CP40	2.31 ± 0.027##	1.56 ± 0.013##	0.80 ± 0.007##

Mean ± Standard error of the mean (n = 6) is how the values are shown. 2000 PCE scored for every animal. Analysis of variance (ANOVA) with a post hoc Dunnett's test

Significant at the **P < 0.001 level when compared to the control group. ##P < 0.001 when compared to cyclophosphamide

Table 2 Analyzing the effect of ELSF in reducing cyclophosphamide-induced DNA damage in mouse bone marrow cells

Treatment (mg/kg)	DNA damage					% of comet
	0	1	2	3	4	
Control (0.9% saline)	96.3	3.54	2.12	-	-	5.12 ± 0.215
Positive control (CP40)	47.2	17.1	18.4	19.6	5.28	45.23 ± 0.223**
ELSF100	97.4	2.53	-	-	-	2.26 ± 0.157**
ELSF200	98.5	2.74	-	-	-	2.64 ± 0.149**
ELSF100 + CP40	81.8	13.2	8.75	4.33	-	24.8 ± 0.475##
ELSF200 + CP40	73.6	15.2	10.26	6.54	-	31.2 ± 0.394##

Mean ± Standard error of the mean (n = 6) is how the values are shown. 100 cells scored for every animal. Analysis of variance (ANOVA) with a post hoc Dunnett's test

Significant at the **P < 0.001 level when compared to the control group. ##P < 0.001 when compared to cyclophosphamide

and 200 mg/kg of the ELSF significantly reduced the comet number with scores presented in Tables 2 and 3. Several parameters of comets (tail length and moment, olive moment and DNA content in the tail) are commonly measured in the alkaline comet experiment to assess the level of DNA damage. Following treatment with cyclophosphamide, it was imperative that there was a statistically significant increase in the DNA fragmentation percentage in BM cells

Table 3 The tail length, tail DNA, tail moment, and olive moment used to investigate ELSF protect mouse bone marrow cells against cyclophosphamide-induced DNA damage

Treatment (mg/kg)	Tail length	Tail DNA percent	Tail moment	Olive moment
Control (0.9% saline)	18.45 ± 0.32	10.05 ± 0.37	2.17 ± 0.08	1.67 ± 0.03
Positive control (CP40)	41.35 ± 2.58**	37.85 ± 3.59**	16.78 ± 2.34**	5.84 ± 1.57**
ELSF100	19.22 ± 0.14**	11.27 ± 0.28**	3.44 ± 0.04**	1.54 ± 0.01**
ELSF200	18.97 ± 0.54**	12.41 ± 0.82**	2.65 ± 0.09**	2.04 ± 0.023**
ELSF100 + CP40	29.32 ± 1.13##	21.34 ± 2.54##	6.76 ± 2.13##	3.77 ± 1.24##
ELSF200 + CP40	32.47 ± 2.17##	27.58 ± 3.01##	8.84 ± 1.87##	4.28 ± 1.18##

Mean ± Standard error of the mean (n = 6) is how the values are shown. 100 cells scored for every animal. Analysis of variance (ANOVA) with a post hoc Dunnett's test

Significant at the **P < 0.001 level when compared to the control group. ##P < 0.001 when compared to cyclophosphamide

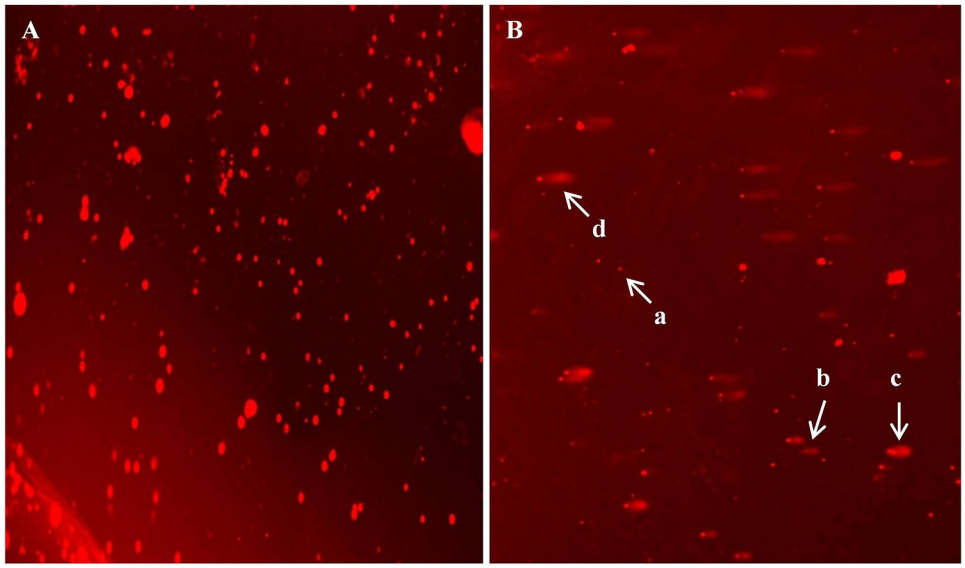


Fig. 3 DNA strand breaks in bone marrow cells caused by cyclophosphamide were detected using the comet assay. **A** Normal cell **B** Cells with mild (a), moderate (b), extensive (c) and complete (d) DNA damage

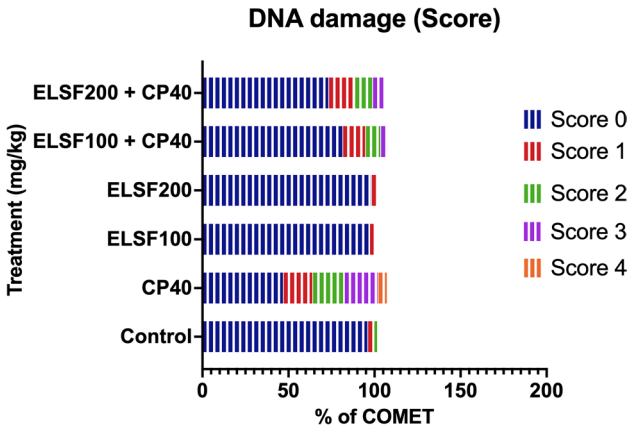


Fig. 4 Analyzing the role of ELSF in preventing DNA damage caused by cyclophosphamide in mouse bone marrow cells. No harm was done; the score was 0. Damage levels from 1 to 4 indicate mild, moderate, high, and severe levels of disruption. Significant decrease in DNA damage in the treatment group was observed

(Fig. 3). The proportion of comets attributable to CP was differed significant in the CP group in response to control group. When particularly in comparison to the CP-treated group, DNA fragmentation was significantly decreased by both doses of ELSF (Fig. 4). However, the marked effect was reflected at low dose ELSF that was equal to 100 mg/kg/bw. The demonstration of underlying mechanistic DNA damage associated with CP and effect on bone marrow cells was elaborated in Fig. 5.

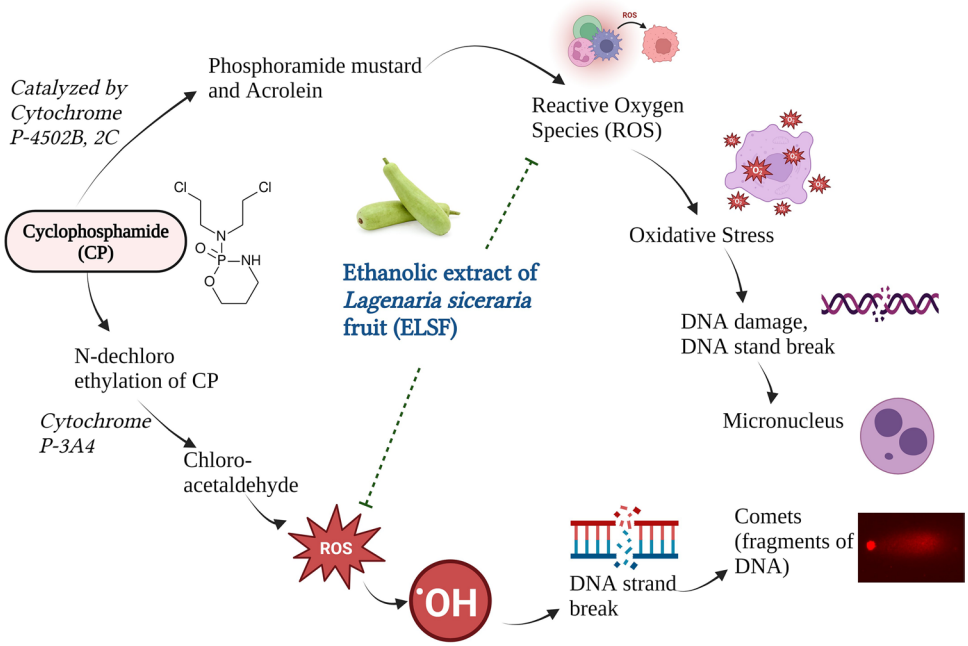


Fig. 5 Detail mechanisms of cyclophosphamide and ELSF

4 Discussion

The current hypothesis is structured to deliver the effect of ELSF (*Lagenaria siceraria* fruit) on the cyclophosphamide-related mechanism of clastogenic/genotoxic potential, thus understanding the potential cause of DNA damage via comet and micronucleus assays. In this research, results clarified that ELSF significantly mitigated the genotoxic effects of cyclophosphamide on BM cells. The two doses were evaluated with significance in reduction by lowest dose. Yet the genotoxic mechanism may be attributed to reduction or neutralization of oxygen radicals by phytochemicals present in *Lagenaria siceraria* fruit. The phytochemicals responsible for the antioxidant activity include Polyphenols, flavonoids, carotenoids and vitamins. The reported constituents such as linolenic acid, β-carotene in the ethanolic extract of *Lagenaria siceraria* fruit have shown promising results in neutralization of free radicals and also involved in bleaching activity to reduce effect of oxygen radicals [22]. These findings are effective to note that the *Lagenaria siceraria* fruit can be a promising agent to treat the chemotherapy related toxic effects mitigating genotoxicity. The CP induced comet number and micronuclei formation has been significantly reduced by ethanolic extract *Lagenaria siceraria* fruit extract at 100 mg/kg dose. Literature supports the anti-clastogenic potential of ELSF against the underlying mechanism of Cyclophosphamide's oxygen/nitrogen free radical production during chemotherapy to its potent genotoxic effect. Deliberately the DNA damage involves with chromosome segregation, DNA damage, or dysfunction of the mitotic spindle can all lead to the development of micronuclei, associated with CP [23].

Spirulina fusiformis has anti-inflammatory, antioxidant, and free radical scavenging properties, as antagonist to the genotoxicity and oxidative damage caused by cyclophosphamide in mice [4]. The effects of ellagic acid, a plant polyphenol, on cyclophosphamide-induced micronuclei and renal oxidative stress in mouse bone marrow cells were investigated by Muneeb et al. [5]. An additional study found that the powerful antioxidant *Foeniculum vulgare* (fennel) essential oil shielded mouse bone marrow cells from CP-induced MN [7]. Recent studies have linked the anti-clastogenic properties of ELSF to its free radical-scavenging properties [18].

There are four levels of damage based on the length of the tail and the percentage of DNA in the tail: undamaged, minimally damaged, moderately damaged, and severely damaged [35]. We found that cyclophosphamide-treated animals had significantly higher comet counts and DNA damage levels in BM cells compared to the controls. Our findings are related to those of Rangel [36] and Tripathi [37], who found a correlation between the number of comets and the degree of DNA damage they cause. The ability of CP to cause DNA damage in mouse bone marrow cells demonstrates its genotoxic potential. There is a correlation between cyclophosphamide's (CP) ability to generate free radicals and the genotoxic effects [38].

Inhibiting transcription, segregation, and replication, cyclophosphamide (also known as cytophosphane) prevents the separation of two strands of DNA by forming a covalent bond between them. Therefore, it kills cells and is cytotoxic [39]. DNA repair enzymes are ineffective at fixing cyclophosphamide-induced DNA adducts and cross-links. Since this is the case, it is possible that the DNA strand break brought on by CP is due to an accumulation of free radicals. The transition pore in the mitochondrial membrane may be affected by the release of free radicals [39]. Most reactive oxygen radicals (ROS) like superoxide anions, hydroxyl ions, peroxide ions, nitric oxide ions, and so on are concentrated in mitochondria, peroxisomes, and cytochrome p450. ROS cause DNA lesions (both single as well as double stand breaks), DNA adducts, cross-links, protein damage, lipid peroxidation, and more. Consequently, the initiation of mitochondrial transition pore allows for cytochrome C release into the cytosol from mitochondria, which in turn activates apoptotic pathway via mitochondria [40].

Results shown that cyclophosphamide causes DNA damage in the erythrocytes of swiss albino mice [41]. This causes free radical production, which in turn damages DNA and kills erythrocytes. DNA damage caused by free radicals has been described in mouse BM cells [42]. In our study, cyclophosphamide showed significant genotoxic effects by increasing the frequency of DNA strand breaks and micronuclei in mouse bone marrow cells. The ELSF coadministration significantly reduced the mutagenicity of cyclophosphamide in a dose-dependent manner, as demonstrated by the MN and comet assays. Two doses of ELSF significantly reduced CP-induced toxicity via chromosomal and DNA damage in mouse BM cells, but ELSF's maximum anticlastogenic effect was only impactful at dose of 100 mg/kg.

The potential of plants and plant components to inhibit cyclophosphamide-induced DNA damage has been studied. Emran reported that the ethanolic extract of *Origanum vulgare* alleviated CP-induced suppression of MN and bone marrow in mouse bone marrow cells [43]. Jena et al. [44] found that the polyherbal formulation (Immu-21) protected bone marrow and peripheral blood cells from the chromosome breaking and myeloma necrosis caused by the chemotherapy drug cyclophosphamide in mice. Total saponins from *Panax ginseng* stem and leaf were studied for their ability to mitigate Cyclophosphamide's toxicity to mouse bone marrow and peripheral lymphocyte cell [45]. In Hepatoma Tissue Culture (HTC) cells, extract from *Casearia sylvestris* reduced the frequency of DNA breaks caused by CP [1]. Damage to DNA caused by cyclophosphamide in mouse peripheral cells of blood was exacerbated by the addition of a saponin- and steroidal compound-rich extract of *Rubus imperialis* [46], which contains niga-ichigoside and tormentic acid. There is speculation that the antioxidant properties of these plants are responsible for their protective effects.

Preserving the free radical/increased antioxidant enzyme activity balance may provide ELSF with protection against the clastogenic effect induced by CP, as suggested by the above results. Various therapeutic benefits of ethanolic extracts of *Lagenaria siceraria* fruit have been previously evaluated [47]. The anticancer effects of *Lagenaria siceraria* seed extracts in acetone were tested in cultures of human breast cancer (MCF-7) and colon cancer (HT-29) cells. The study research indicates that wild bottle gourd is rich in bioactive metabolites with potential anti-cancer, anti-diabetic, and anti-acetylcholine esterase effects [48]. The antioxidant and hypoglycemic effects of an ethanolic extract of bottle guard fruits were studied [47], at 40 mg/kg dose in glucose-induced hyperglycemic mice was essentially equivalent to glibenclamide. The antioxidant activity of the ethanolic extract was measured using the ferric reducing/antioxidant power assay (FRAP), the DPPH free radical scavenging assay, and the chelating power. Antioxidant and anti-hyperglycemic properties were also observed [49]. Iqra Atique et al. also reported these benefits, including their anti-diabetic and antioxidant properties [18, 50]. Antia et al. reported that *Lagenaria siceraria* seed extracts have significant amounts of polyphenolic compounds and strong antioxidant activity [51], and a root extract of *Lagenaria siceraria* was found to have potent analgesic activity in albino mice when administered at doses of 100 mg/kg and 200 mg/kg via the tail flick method. His research lends credence to the idea that these seeds, which have previously been put to use mainly for edible oils and to thicken soup, are rich in antioxidants and may have other biological uses as well [47]. Comparable to other species of same family such as *Citrullus colocynthis* (L.) fruit (ethanolic extract) has shown marked chemoprotective effect owing to mitigated bone marrow suppression at 200 mg/kg [52]. Limited literature was available on the same family. The phenolic compound is responsible with high antioxidant potential, ethanolic extracts of *Lagenaria siceraria* fruit have not shown a significant statistically frequency of MN strand breaks at any of the tested doses. Antioxidant activity made it clear that the lower of the DNA two ELSF doses (100 and 200 mg per kg/bw) in combination with cyclophosphamide was more effective. We can attribute this to the fact that ELSF's antioxidant activity is most potent at lower concentrations, where it produced the greatest effect. Polyphenolic compounds are found naturally in vegetables, fruits, and various other plant parts. Possible positive biological effects include free radical scavenging and antioxidant effects. Based on the, a fore mentioned literature, we concluded that two doses of ELSF have potent cyclophosphamide-induced anticlastogenic activity.

5 Conclusion

Lagenaria siceraria fruit was investigated for various pharmacological activities, literature supports no findings on genotoxicity. The ELSF was reported to be enriched with Vitamins A, C, and E, carotenoids, polyphenolic substances, and flavonoids are responsible for chemoprotective activity. The doses were selected based upon supporting literature and the hypothesis was framed to investigate Anticlastogenic activity which was not reported earlier. Anticlastogenic potential estimates the DNA formation involving cyclophosphamide pathway. Thus, comet and micronuclei assay help in investigate the underlying mechanisms of DNA damage. Our research proved that ethanolic extracts in doses of 100 and 200 mg/kg (*Lagenaria siceraria* fruit) protected Swiss albino mouse bone marrow cells from cyclophosphamide-induced genotoxicity. The underlying mechanism is due to presence of free radical scavenging flavonoids and polyphenolics. A key component in lowering the frequency of DNA strand breaks caused by cyclophosphamide, involves neutralization and complexation by antioxidant rich ELSF. The future investigations may be researched on the in vivo mechanisms of genotoxicity for ELSF's protective role against oxidative stress-induced DNA damage and its ability to scavenge free radicals. The dose characteristics were reported limited for 100 mg/kg and 200 mg/kg, further escalation of doses can be estimated and reported as future research findings.

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Author contributions All authors made contributions to the conception and design of the investigation. Rajini Kolure, Naveen Reddy Penumallu, Sneha Thakur, Somnath De, Suhasini Boddu, Nachammai Vinaitheerthan, Ramreddy Godela, and Manjula Santhepete Nanjundaiah were responsible for material preparation, data collection, and analysis. Rajini Kolure authored the initial draft of the manuscript, and all authors provided feedback on interim versions of the document. The final manuscript was reviewed and endorsed by all authors.

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Data availability The corresponding author can be reached for a reasonable request for the datasets used and/or analysed in the current study.

Code availability Not applicable.

Declarations

Ethics approval All the permissions for the ethical consent to perform the experiment were approved in CPCSEA meeting. The protocol was approved and experiment was performed within premises of central facility, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India as per OECD guidelines.

Consent to participation Not applicable.

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