Natural Resources for Human Health

eISSN: 2583-1194

Original Research

Received 28 May 2026
Revised 22 June 2026
Accepted 17 July 2026

Natural Resources for Human Health 2026, Vol. 6 (10s): 814–828

KEYWORDS:
Annona muricata , plant waste; cytotoxicity; cytogenotoxicity; cyclophosphamide.

Comparative Evaluation of the Cytogenetic Effects of *Annona spp*. Peel and Seed Extracts in In Vitro and In Vivo Models

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ABSTRACT: In recent years, there has been a growing interest in assessing industrial agricultural plant residues as potential sources of bioactive compounds with therapeutic value. Accordingly, the present study aimed to determine the cytogenetic effects of extracts derived from the peel and seeds of soursop (*Annona muricata*) in male albino rats (in vivo) and in two cancer cell lines (in vitro), The findings indicated that extracts from the soursop fruit peel and seeds reduced cell viability in a manner dependent on both *concentration* and exposure time.

The inhibitory effects reached their maximum 72 hours following treatment at the highest experimental concentration of 1000 µg/mL Using the cytotoxicity data, an optimal concentration was selected for each extract, characterized by minimal cytotoxicity and the maintenance of cell viability near the negative control level. These concentrations were subsequently used to assess cytotoxic and genotoxic effects and to evaluate the protective action against cyclophosphamide. Cytotoxicity assays demonstrated that cyclophosphamide exposure produced significant alterations in cellular and genetic indicators—specifically, a reduction in the mitotic index and an elevation in genetic damage markers relative to the negative control. Conversely, co-treatment with the optimal concentrations of peel and seed extracts alongside cyclophosphamide improved these parameters, as evidenced by an increased mitotic index and decreased genetic damage markers compared with the group treated with cyclophosphamide alone. Collectively, the results indicate that the two extracts, when administered at non-toxic or low-toxicity concentrations, exert protective effects at the cellular and genetic levels against cyclophosphamide-induced injury.

Moreover, basing the selection of the optimal concentration on the cytotoxicity results is important for interpreting this protective effect: the improvement in the mitotic index and the reduction in genetic damage markers at that concentration suggest that protection is not merely a consequence of cytotoxicity, but may instead be associated with the extracts' capacity to counteract the harmful effects of cyclophosphamide.

1. INTRODUCTION

Agricultural and food waste has become a major issue of increasing concern in recent years due to its environmental and economic repercussions when disposed of improperly (Adejumo and Adebiyi 2020; Lackner and Besharati, 2025). However, numerous studies have shown that a significant proportion of this waste contains valuable compounds that can be utilized in various fields, making its reuse an important option within the framework of sustainability and the circular economy (Tchonkouang et al, 2023; Pandey et al, 2025).

Fruit peels are among the most abundant types of agricultural waste, produced in large quantities as a result of direct consumption or during food processing (Islam et al., 2025). Research indicates that these peels are not merely byproducts, but also contain active compounds such as phenolic compounds, flavonoids, alkaloids, and antioxidants, which are linked to several biological activities, including resistance to oxidation, inflammation, and microbes, in addition to their potential effects in reducing the growth of cancer cells (Singh et al. 2025; Ouro-Salim et al,2026).

One plant that has garnered significant research attention in recent years is the soursop (*Annona muricata L.*) due to the diverse bioactive compounds found in its fruit, leaves, seeds, and peel. These studies have focused on the use of all aerial parts of the plant, including bark, leaves, roots and seeds, as natural medicines in tropical regions (Zubaidi et al., 2023; Ramos et al., 2024). In contrast, research has shown limited importance for peels. Although the peel is usually thrown away after consuming the fruit, it represents a rich source of active ingredients that may contribute to future medical applications, allowing for its use instead of disposal (Ojo et al., 2022; Zubaidi et al., 2023).

Meanwhile, cancer remains one of the most prevalent health problems worldwide, associated with high mortality male albino mice and increasing health and economic burdens (Boussios et al., 2024; Ekhlas, 2026). As treatment methods continue to evolve, the limited effectiveness of some therapies and their associated side effects have prompted researchers to explore natural sources that could contribute to the development of safer alternatives or adjuvant agents (Sani et al., 2025). In light of this, numerous studies have focused on evaluating the biological activity of plant extracts (Zouine et al, 2025; Budiman, et al, 2024), particularly those derived from agricultural waste, due to their potential to influence cancer cell growth and survival mechanisms. Therefore, this study aims to assess the anticancer activity of soursop peel extracts and highlight the potential of utilizing this agricultural by product as a natural source of compounds with possible pharmaceutical value. This approach promotes the sustainable use of plant resources and supports the move towards the sustainable use of agricultural waste.

2. MATERIALS AND METHODS

2.1 Plant Collection:

The fruit of *A. muricata* was obtained from local markets in Baghdad. The peels were separated from the fruit and air-dried. The seeds were also isolated from the fruit and air-dried at room temperature. The plant parts were ground separately using an electric grinder to obtain a fine powder. This powder was then passed through a 40-micrometer sieve and stored in airtight glass containers until extraction.

2.2 Preparation of the Extract

Aqueous extraction of peels and seeds of *A. muricata* was performed by soaking 100 g of each powdered sample in 1000 mL of distilled water (1:10, w/v) for 12 hours. The extracts were filtered separately using Whitman filter paper and used in subsequent studies. Each sample was concentrated individually under low pressure using a rotary evaporator at 40-45°C until approximately one-tenth of the original volume remained (Sulistyoningrum et al., 2017). The concentrated extracts were dried in a vacuum oven at 40 °C until weight constant.

2.3 Effects of isolated seed and peel of *A. muricata* in vivo

2.3.1 Laboratory Animals

The current study experiments were conducted on male albino mice (Mus musculs) supplied from the animal house of the Biotechnology Research Center /Al-Nahrain University. The animals were 9-10 weeks old at the start of the experiments and weighed between 24-28 grams. The animals were divided into plastic cages according to the needs of the experiments and placed in the animal house of the Biotechnology Research Center /Al-Nahrain University at a temperature between 20-25°C and a lighting schedule of 14 hours of light and 10 hours of darkness. They were given locally manufactured feed (pellets) and free-range water.

The experiment was conducted to determine the optimal dosage of aqueous extracts of peel and seed of *A. muricata* by evaluating their safety and biological effects on healthy male albino mice. This experiment used 42 male albino mice, with 6 mice in each group.

- Group 1 (Negative Control): Mice received distilled water only (n=6)

- Group 2 (Positive Control 1): Mice received cyclophosphamide (40 mg/ kg^{-1} bw) (n=6)
- Group 3 (Positive Control 2): Mice received vitamin C (180 mg/ kg^{-1} bw) (n=6)
- Groups 4: Mice received peel of *A. muricata* extract (200 mg/ kg^{-1} bw) by oral gavage (0.1 ml) once daily for seven consecutive days (n=6)
- Groups 5: Mice received peel of *A. muricata* extract (400 mg/ kg^{-1} bw) by oral gavage (0.1 ml) once daily for seven consecutive days (n=6)
- Groups 6: Mice received seed of *A. muricata* extract (200 mg/ kg^{-1} bw) by oral gavage (0.1 ml) once daily for seven consecutive days (n=6)
- Groups 7: Mice received seed of *A. muricata* extract (400 mg/ kg^{-1} bw) by oral gavage (0.1 ml) once daily for seven consecutive days (n=6)

While the animals were sacrificed on the eighth day for the purpose of conducting the required laboratory tests.

2.3.2 Protective Effect of Peel and Seeds of *A. muricata*

In this phase, 27 male albino rats were used and randomly divided into four groups

- Group 1: (Positive Control): Mice received cyclophosphamide (40 mg/ kg^{-1} bw) (n=6)
- Group 2: Mice received a single intraperitoneal injection of cyclophosphamide (40 mg/ kg^{-1} bw) on Day 1. On the same day, treatment with vitamin C (180 mg/ kg^{-1} bw) was initiated by oral gavage and continued once daily until the designated sacrifice time (n=6)
- Groups 3: Mice received a single intraperitoneal injection of cyclophosphamide (40 mg/ kg^{-1} bw) on Day 1. On the same day, treatment with peel of *A. muricata* extract (200 mg/ kg^{-1} bw) was initiated by oral gavage and continued once daily until the designated sacrifice time (n=6)
- Groups 4: Mice received a single intraperitoneal injection of cyclophosphamide (40 mg/ kg^{-1} bw) on Day 1. On the same day, treatment with seed of *A. muricata* extract (200 mg/ kg^{-1} bw) was initiated by oral gavage and continued once daily until the designated sacrifice time (n=6)

The animals were sacrificed on the eighth day for the required laboratory tests.

2.4 Cytogenetic Parameters

2.4.1 Mitotic Index

After the experiment, mice were injected intraperitoneally with 0.25 mL of colchicine solution two hours before sacrifice to stop cell division in metaphase. Bone marrow cells were collected from the femur by washing with 5 mL of warm physiological saline. Tubes containing bone marrow cells were centrifuged at 1000 rpm for 10 minutes, then treated with hypotonic potassium chloride solution (0.075 M) and incubated in a water bath (37°C) for 30 minutes with shaking every 5 minutes. The cells were centrifuged at 1000 rpm for 10 minutes, then the supernatant was discarded, and the precipitated cells were resuspended in a freshly prepared, cold fixative solution. The fixative process was repeated twice, and the cell precipitate was resuspended in 1-2 mL of the fixative solution. The cell suspension was dropped onto clean glass slides and allowed to air dry. The slides were then stained with Cemsa stain for 15 minutes, washed with distilled water, allowed to dry, and examined under a light microscope at 40X magnification. The cell division index was calculated by examining at least 1000 cells per animal under a light microscope at 1000X magnification according to the following equation:

Mitotic Index (%) = (Number of Dividing Cells ÷ Total Number of Cells) × 100

2.4.2 Chromosomal Aberration Assay

Chromosomal aberrations were assessed using metaphase spreads prepared from bone marrow cells. At least 100 metaphase cells from each animal were examined using a light microscope at 1000x magnification (Ekhlas , 2014).

2.4.3 Micronucleus formation

Micronucleated erythrocytes (MNPCEs) were examined on bone marrow smears prepared immediately after cell collection. The smears were then allowed to air dry, fixed in absolute methanol for 10 minutes, and the slides were left to dry. They were

then stained with Camsa stain for 20 minutes and allowed to dry. Two thousand polychromatic erythrocytes (PCEs) per animal were examined using a light microscope at 1000× magnification, and the number of cells containing micronucleated polychromatic erythrocytes (MNPCEs) was recorded according to the following equation.

Micronucleus formation index (%) = (Number of micronuclei ÷ Total number of cells) × 100

2.5 Cytotoxicity Assay

Using two cancer cell lines, MDA-MB-231 (breast cancer) and HepG2 (liver cancer), obtained from the Biotechnology Center/Al-Nahrain University. Extracts were prepared according to Freshney, (2021) by dissolving 0.2 g of crude extract powder of *Annona muricata* peel and seeds separately in 10 ml of phosphate buffer solution. The dissolved extract was then sterilized using a 0.22 µm perforated filter, and eight concentrations were prepared for each sample by two-fold dilution using serum-free medium (78, 125, 156, 25, 312, 5, 62, 125, 250, 5000, 10000) µg/ml under sterile conditions. All prepared concentrations were used immediately after their preparation.

Cell suspensions were prepared by treating cultured cells with trypsin-EDTA solution. A culture medium containing 10% serum was added, and 0.2 ml of the cell suspension was transferred to the wells of 96-well flat-bottomed cell culture plates. The plates were incubated at 37°C for 12–18 hours until cell adhesion occurred. The medium was then replaced with 0.2 ml of extracts at different concentrations, with four replicates per concentration, including a control group of four replicates (Feoktistova et al, 2016).

After the exposure period, the culture medium was removed, and the cells were washed with PBS. 0.1 ml of crystal violet was then added to each well and left to stand for 20 minutes. The cells were then washed repeatedly with PBS until excess stain was removed. After the plates had completely dried, the plates were analysed by using an ELISA microplate reader at 492 nm. The exposure periods were 24, 48, and 72 hours. Each treatment was performed in triplicate, and the entire experiment was independently repeated three times. (Ziemba et al., 2025)

Cell viability was calculated using the following equation:

Cell viability (%) = (Absorbance of treated cells / Absorbance of untreated control cells) × 100

The percentage of growth inhibition was calculated as:

Growth inhibition (%) = 100 − Cell viability (%)

2.6 Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference groups in study parameters. Least significant difference-LSD was used to significant compare between means in this study.

3. RESULTS AND DISCUSSION

3.1 Cytogenetic effects of apple and seed of *A. muricata* in Mitotic Index (MI)

The results shown in Table 1 indicate a significant decrease in the cell division coefficient of animals treated with cyclophosphamide 2.26%, compared with the negative control 7.28% and the second positive control (mice treated with vitamin C 8.10%). this difference constituted a significant difference at the probability level P≤0.01. In contrast, the treatment of laboratory male albino mice with extracts of the peel and seeds of *A. muricata* alone at the doses of 400 and 200 mg/ kg^{-1} bw did not show any significant effect compared to the negative control group P≤0.01. Figure 1

Table 1. Mitotic index of bone marrow cells in male albino mice treated with *A. muricata* seed and peel extract

Group	Mean ±SE
Normal control	7.28 ±0.03 c
Cyclophosphamide (40 mg/ kg^{-1} bw)	2.26 ±0.07 g

C (Ascorbic acid) (180 mg/ kg^{-1} bw)	8.10 ±0.06 a
A. muricata seed (200 mg/ kg^{-1} bw)	8.23 ±0.13 a
A. muricata seed (400 mg/ kg^{-1} bw)	7.46 ±0.06 c
A. muricata peel (200 mg/ kg^{-1} bw)	8.15 ±0.07 a
A. muricata peel (400 mg/ kg^{-1} bw)	7.86 ±0.08 b
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

3.2 Cytogenetic effects of apple and seed of *A. muricata* in Chromosomal Aberration

The results shown in Table (2) demonstrate a significant increase in the percentage of chromosomal abnormalities in bone marrow cells of the cyclophosphamide-treated control group, reaching 8.57%, compared to 1.26% in the negative control group and 1.28 % in vitamin C. This increase was statistically significant at the significance level (P≤0.01). In contrast, the two doses (200 and 400 mg/ kg^{-1} bw) of *A. muricata* peel and seed extracts did not induce chromosomal abnormalities in the bone marrow cells of the mice treated with them. Although a slight increase in the percentage of abnormalities was observed in some groups compared to the negative control group, this increase was not statistically significant (P≤0.01) compared to the positive control group, indicating that the plant extracts used do not possess mutagenic or aberrant effects under the current study doses and conditions.

Table 2. Representative Analytical Results

Group	Mean ±SE
Normal control	1.26 ±0.12 f
Cyclophosphamide (40mg/ kg^{-1} bw)	8.57 ±0.24 a
C (Ascorbic acid) (180 mg/ kg^{-1} bw)	1.28 ±0.08 ef
A. muricata seed (200 mg/ kg^{-1} bw)	1.42 ±0.06 ef
A. muricata seed (400 mg/ kg^{-1} bw)	3.42 ±0.34 d
A. muricata peel (200 mg/ kg^{-1} bw)	1.76 ±0.04 e
A. muricata peel (400 mg/ kg^{-1} bw)	4.14 ±0.15 c
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

3.3 Cytogenetic effects of apple and seed of *A. muricata* in Micronucleus

The results shown in Table 3 indicate that treatment of mice with cyclophosphamide (positive control group) resulted in a significant increase in the rate of micronucleus formation in multicolored red blood cells, reaching 9.56%, compared to 1.33% in the negative control group and 2.62% in vitamin C. This increase was statistically significant at the significance level (P ≤ 0.01). However, treatment of mice with extracts of the bark and seeds of *A. muricata* at doses of 200 and 400 mg/ kg^{-1} bw respectively, did not show any significant increase in the rate of micronucleation compared to the negative control group (P > 0.01). Although a slight increase was observed in some groups, this increase did not reach statistical significance, indicating that the plant extracts used, at the doses studied, and did not exhibit a mutagenic effect leading to micronucleation. .

Table 3. Micronucleus frequency in bone marrow cells of male albino mice treated with *A. muricata* seed and peel extract

Group	Mean ± SD
Normal control	1.33 ±0.02 h
Cyclophosphamide (40 mg/ kg^{-1} bw)	9.56 ±0.17 a
C (Ascorbic acid) (180 mg/ kg^{-1} bw)	2.62 ±0.07 f
A. muricata seed (200 mg/ kg^{-1} bw)	2.92 ±0.14 f
A. muricata seed (400 mg/ kg^{-1} bw)	4.51 ±0.06 d
A. muricata peel (200 mg/ kg^{-1} bw)	1.78 ±0.11 g
A. muricata peel (400 mg/ kg^{-1} bw)	3.34 ±0.19 e

3.4 Protective Effects of Annona muricata Peel and Seed Extracts on Mitotic Index

The results in Table 4 showed a significant increase in the cell division coefficient in mice treated with the optimal dose of peel 6.85 %and seeds 6.59 % respectively, compared to the negative control (2.26 %) and also in mice treated with vitamin C (5.13 %). The difference was significant in both treatments Figure 1

Table 4. Mitotic index of bone marrow cells in male albino mice co-treatment with optimal dose of *A. muricata* seed and peel extract and cyclophosphamide

Group	Mean ± SD
Cyclophosphamide (40 mg/ kg^{-1} bw)	2.26 ±0.07 g
C (Ascorbic acid) (180 mg/ kg^{-1} bw) + Cyclophosphamide (40 mg/ kg^{-1} bw)	5.13 ±0.09 f
A. muricata seed (200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	6.59 ±0.07 e
A. muricata peel (200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	6.85 ±0.07 d
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

3.5 Protective Effects of *A.muricata* Peel and Seed Extracts on Chromosomal aberration (CA)

The results shown in Table 5 showed a significant decrease in the percentage of chromosomal abnormalities in the group of mice treated with extracts of *A. muricata* peels 3.25 % and seeds 4.23% respectively compared to the negative control (8.57 %). Vitamin C also caused a significant decrease (6.45%) compared to the negative control. However, we note that the decrease in the percentage of chromosomal abnormalities in the group treated with peels and seed extracts of *A. muricata* was better than the treatment with vitamin C. Figure 1

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eISSN: 2583-1194

Table 5. Chromosomal aberration frequency of bone marrow cells in male albino mice co-treatment with optimal dose of *A. muricata* seed and peel extract and cyclophosphamide

Group	Mean ± SD
Cyclophosphamide (40 mg/ kg^{-1} bw)	8.57 ±0.24 a
C (Ascorbic acid) (180 mg/ kg^{-1} bw) + Cyclophosphamide (40 mg/ kg^{-1} bw)	6.45 ±0.02 b
A. muricata seed (200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	4.23 ±0.15 c
A. muricata peel (200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	3.25 ±0.15 d
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

3.5 Protective Effects of Annona muricata Peel and Seed Extracts on Micronucleus (MN)

The results shown in Table 6 indicate a significant decrease (P≤0.01) in the micronucleus index in mice treated with the optimal dose of Peel and seed (4.67%, 5.37% respectively), compared to the negative control group (9.56%) and the vitamin C group (6.96%). This decrease was significant in both treatments (Figure 1).

Table 6: Micronucleus frequency of bone marrow cells in male albino mice co-treatment with optimal dose of *A. muricata* seed and peel extract and cyclophosphamide

Group	Mean ± SD
Cyclophosphamide (40 mg/ kg^{-1} bw)	9.56 ±0.17 a
C (Ascorbic acid) (180 mg/ kg^{-1} bw) + Cyclophosphamide (40 mg/ kg^{-1} bw)	6.96 ±0.16 b
A. muricata seed(200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	5.37 ±0.09 c
A. muricata peel(200 mg/ kg^{-1} bw) + CP (40 mg/ kg^{-1} bw)	4.67 ±0.02 d
Means having with the different letters in same column differed significantly. ** (P≤0.01).	

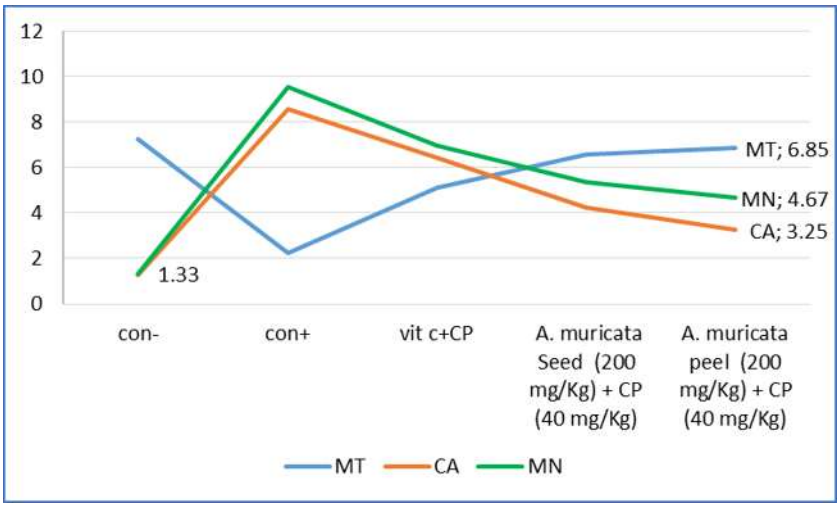

Figure 1. Effects of co-treatment with the optimal dose *A.muricata* extract and cyclophosphamide on mitotic index, micronucleus frequency, and chromosomal aberrations in male albino mice.

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3.6 Toxicity effect of Aqueous Extract of Seeds and Peels of *A. muricata*, in Cancer Cell Lines

Hep-2 Human laryngeal carcinoma cell line

Statistical analysis of Hep-2 liver cancer cell survival mice after exposure to 10 different concentrations of aqueous extracts of *A. muricata*, seeds and peels for 48 and 72 hours showed a significant decrease in cell viability that depended on the duration of exposure and increasing concentration. The highest inhibition of cell viability was recorded at a concentration of 2000 µg/ml for both the peel and seed extracts of *A. muricata*, as shown in Table 7. Cell survival rates were 28.71 % and57.68 %, respectively, after 48 hours of incubation. The toxic effect increased when cells were exposed to the same concentrations for a duration of 72 hours, showing statistically significant differences ($P \leq 0.01$) of 6.37 % and 34.92 %, respectively, after 72 hours of incubation. The lowest concentration, 3.906% µg/ml, did not produce any statistically significant differences compared to the control group (Figure 2, 3).

Table 7: Effect of different concentrations of A. muricata Peel and seed extract on the viability of HEp-2 cells after 48, and 72 h

Concentrations (µg/ml)	Seed extracts Mean ±SE		Peel extracts Mean ±SE	
	IR%		IR%	
	48	**72**	**48**	**72**
0	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a
3.906	98.8 ±1.06 ab	98.4 ±1.15 ab	99.5 ±0.63 a	99.89 ±0.46 a
7.8125	92.54 ±3.17 bc	90.65 ±3.44 bc	90.9 ±2.36 b	89.2 ±3.51 b
15.62	91.83 ±3.06 bcd	89.56 ±3.61 c	89.56 ±3.76 b	82.84 ±3.48 bc
31.25	89.5 ±3.16 cd	80.87 ±3.29 d	77.86 ±3.55 c	75.85 ±3.50 c
62.5	84.86 ±3.75 de	74.42 ±2.86 de	69.5 ±3.01 cd	65.45 ±3.19 d
125	77.96 ±3.02 ef	68.88 ±3.15 ef	61.78 ±2.47 de	53.19 ±2.26 e
250	74.57 ±2.91 fg	61.86 ±2.75 fg	59.18 ±2.75 e	38.43 ±2.50 f
500	66.87 ±2.48 gh	58.12 ±2.35 g	43.36 ±2.12 f	22.38 ±0.85 g
1000	60.55 ±2.41 hi	41.46 ±2.07 h	39.8 ±1.84 fg	14.93 ±0.72 h
2000	57.68 ±1.95 i	28.71 ±1.26 i	34.92 ±1.25 g	6.37 ±0.37 h
Means having with the different letters in same column differed significantly** (P≤0.01).				

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eISSN: 2583-1194

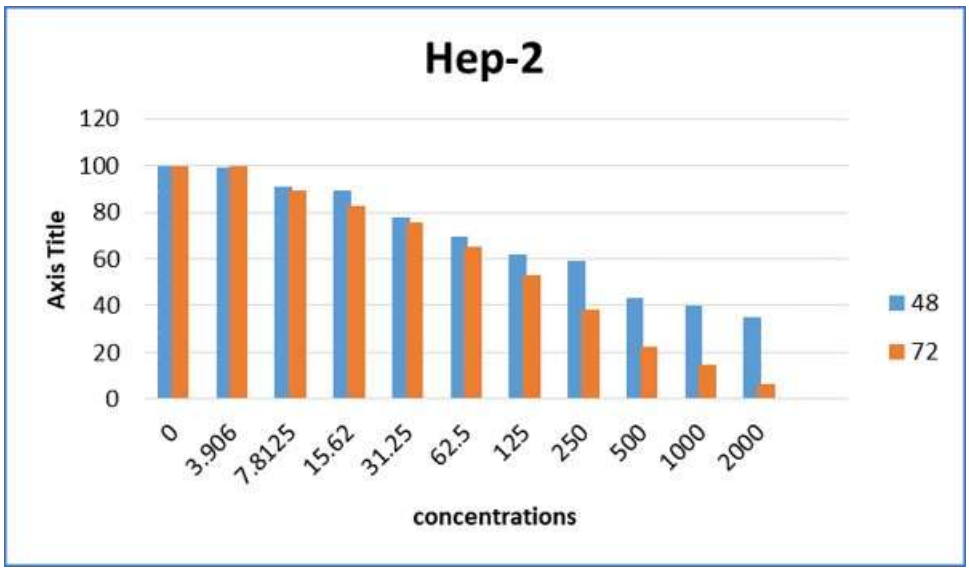

Figure 1 Comparison of the effects of different concentrations of A. muricata peel extracts on Hep-2 cell viability after 48, and 72 h.

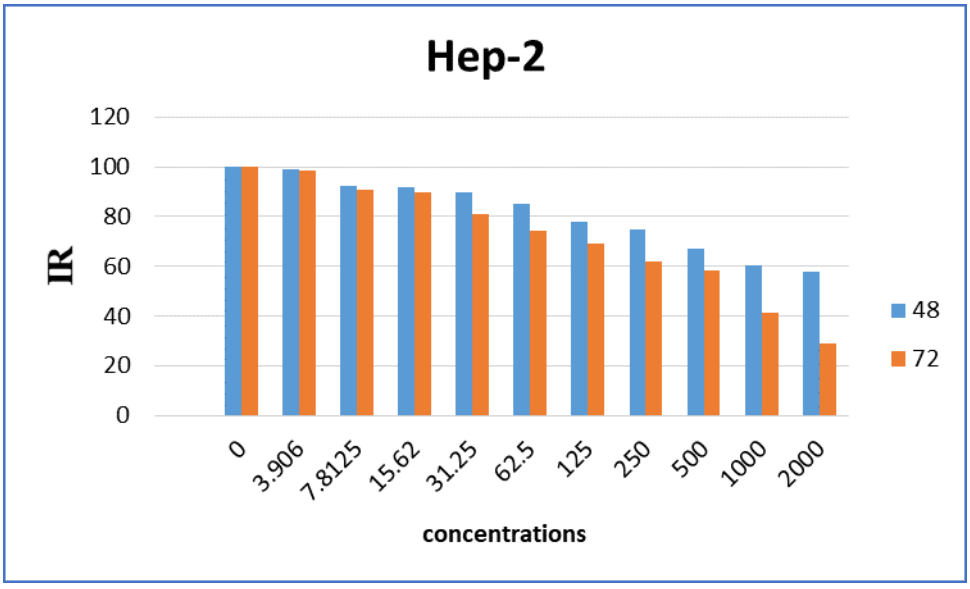

Figure 2 Comparison of the effects of different concentrations of A.muricata Seed extracts on HEp-2 cell viability after 48, and 72 h

3.7 Toxicity effect of Aqueous Extract of Seeds and Peels of *A. muricata*, in AMN-3

The results shown in Table 8 indicate a high cytotoxic effect when extracts of *A. muricata* seeds and peel at different concentrations were used on the AMN-3 mouse gland cancer cells. This effect was significant compared to the control group after incubation periods of 48 and 72 hours (Figure 2). A significant decrease in AMN-3 cell viability was also observed at a concentration of 2000 µg/ml ($p \leq 0.01$). Cell survival rates after 48 hours of treatment with PE and SE were 50.0 % and 53.2 %, respectively. Inhibition rates then increased, reaching their maximum with an incubation period of 72 hours, at which cell survival rates were 21.47 % and 33.5 %, respectively. (Figure 4, 5)

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Table 8. Effect of different concentrations of *A.muricata* Peel and seed extract on the viability of AMN-3 cells after 48, and 72 h

Concentrations (µg/ml)	Seed extracts Mean ±SE		Peel extracts Mean ±SE	
	IR%		IR%	
	48	**72**	**48**	**72**
0	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a
3.906	99.5 ±0.52 a	99.3 ±0.54 ab	99.2 ±0.41 a	92.7 ±3.18 ab
7.8125	98.5 ±0.68 ab	95.5 ±1.81 abc	92.5 ±2.86 ab	89.6 ±3.53 bc
15.62	92.86 ±3.15 abc	90.0 ±3.78 bc	88.2 ±3.56 bc	82.0 ±3.19 cd
31.25	90.77 ±3.63 bc	87.5 ±3.44 cd	84.87 ±3.20 bcd	77.5 ±3.55 de
62.5	88.78 ±3.07 cd	79.84 ±.26 de	82.0 ±2.97 cd	71.86 ±2.89 ef
125	84.6 ±2.92 cd	71.45 ±1.16 ef	77.6 ±3.48 de	64.84 ±2.92 f
250	80.2 ±2.84 d	66.45 ±2.08 fg	71.4 ±3.22 ef	54.7 ±2.44 g
500	71.55 ±3.19 e	57.74 ±2.31 g	63.6 ±2.87 fg	43.86 ±2.86 h
1000	67.1 ±2.66 e	42.0 ±1.87 h	59.7 ±2.17 gh	39.75 ±2.05 h
2000	50.0 ±1.94 f	33.5 ±1.25 h	53.2 ±1.66 h	21.47 ±0.74 i
Means having with the different letters in same column differed significantly** (P≤0.01).				

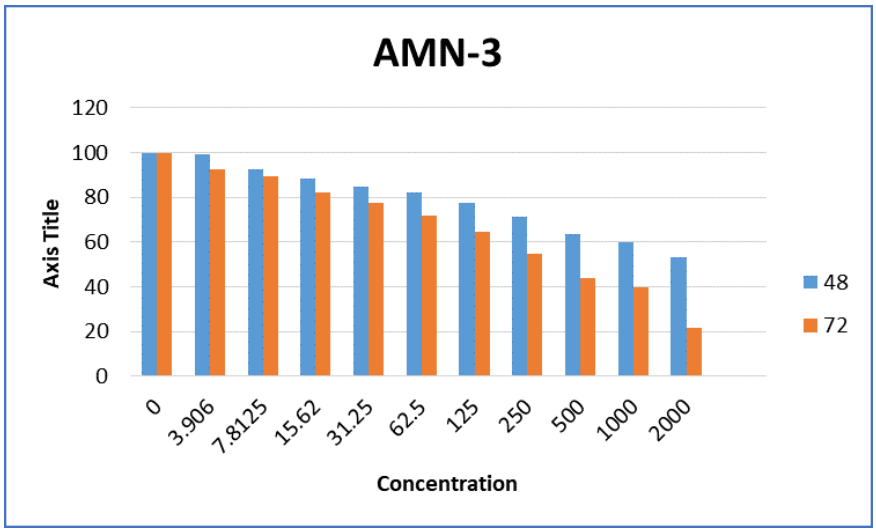

Figure 4. Comparison of the effects of different concentrations of A. muricata peel extracts on AMN-3 cell viability after 48, and 72 h

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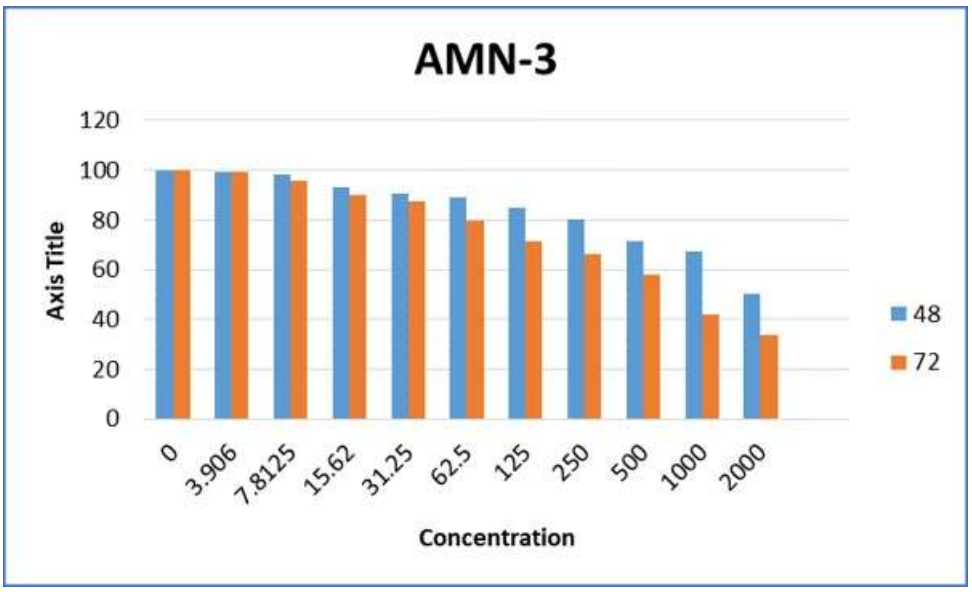

Figure 5. Comparison of the effects of different concentrations of *A. muricata* seed extracts on HEp-2 cell viability after 48, and 72 h

4. DISCUSSION

Agricultural waste and related industrial byproducts constitute a major source of accumulated organic matter. Unsustainable handling of this waste exacerbates pollution problems and wastes potentially valuable resources. However, this waste is rich in diverse organic and bioactive components such as cellulose, hemicellulose, lignin, and phenolic compounds, making it a promising raw material for producing numerous value-added products. Therefore, many studies have sought to redefine agricultural waste as an investment resource rather than an environmental burden, particularly through its use in food production, bio-products, or fungal culture media.

The current study demonstrated that cyclophosphamide contributed to increased chromosomal aberrations and micronuclei, as well as a decreased mitotic index. This may be due to the conversion of cyclophosphamide (known to be an alkylating agent) to phosphoramide and acrolein, forms resulting from its metabolic transformation. These metabolites react with oxygen to produce reactive oxygen species, causing oxidative stress, DNA strand breaks, and chromosomal instability. This ultimately leads to an increase in chromosomal aberrations and micronuclei, as well as inhibition of mitotic activity (Mills et al., 2019; Hirshman et al, 2020; Ekhlas, 2026).

In contrast, the results of the genotoxicity assessment showed that 200 and 400 mg/ml of the aqueous extract of the peels and seeds of *A. muricata* did not cause any significant change in the values of the cytokinesis index, chromosomal aberrations, or micronucleus frequency compared to the cyclophosphamide group, and were close to the values of the negative control group and vitamin C (as known as an antioxidant properties). This indicates it's no genotoxicity and the absence of genotoxic or mutagenic effects at the bone marrow cell level. These effects is attributed to the antioxidant properties present in the peels and seeds of *A. muricata*, as indicated by the studies of Ilango et al. (2022) and Bhosale et al. (2021). In light of these results, a lower dose of 200 mg/ml of each extract was adopted as the optimal dose for cyclophosphamide interference trials, as this dose did not cause any toxic effects on bone marrow cell genes.

To investigate the protective role of custard apple peels and seeds, a co- treatment with cyclophosphamide was used. To explore the protective effects of custard apple peels and seeds, a co-treatment with cyclophosphamide was conducted. Findings showed that an optimal dose of each extract, administered separately alongside cyclophosphamide, reduced the cytotoxic effects of the drug and significantly improved all cellular indicators compared to the group receiving only cyclophosphamide. Rates of chromosomal abnormalities and micronucleus formation decreased, while the mitotic index increased relative to the control group. Both peel and seed extracts altered these ratios to levels close to or even surpassing normal values. They often demonstrated greater antimutagenic activity than vitamin C. These findings suggest that the aqueous extract of *A. muricata* peel

and seeds has cytogenetic protective activity, thanks to the presence of bioactive compounds like flavonoids and tannins, known for their antioxidant properties and capacity to reduce oxidative stress (González-Pedroza et al, 2021; Abbood et al, 2025). These compounds help protect DNA from damage caused by cyclophosphamide metabolites. They may inhibit mutagenic activity by forming complexes with the mutagen or its metabolites, preventing their cellular entry. Alternatively, the antimutagenic compound might interact with the mutagen's target sites by acting as a barrier, hindering the mutagen from accessing or interacting with its target DNA sites, thus mitigating its mutagenic effects (Sloczyńska, et al, 2014 ; Arhoghro et al,2023) .

The in vitro study results indicated that treating the cancer cell lines Hep-2 and AMN3 with varying concentrations of extracts from soursop fruit (*Annona muricata*) seeds and peels, ranging from 7.8125 to 2000 micrograms/ml for 48 to 72 hours, significantly reduced cancer cell growth and survival rates. The effectiveness depended on both the concentration and exposure duration. The peel extract proved more potent than the seed extract in reducing cell viability across the treatment periods. This increased efficacy is linked to the bioactive compounds present in the peel, such as soursop acetogenins, phenols, flavonoids, and tannins, which collectively influence multiple cellular pathways by triggering apoptosis and limiting cell proliferation (Rady et al, 2018; Qazi et al, 2018). This enhances the anticancer effects while increasing selective toxicity against cancerous cells compared to normal ones (Silihe et al,2023),. These observations align with the findings of Ilango et al. (2022), who reported that crude soursop extracts display cytotoxicity against several cancer cell lines, including breast cancer (MCF-7 and MDA-MB-468), colon cancer (HCT-116), and melanoma (A-375), as well as phagocytic cell lines, underscoring the plant's broad-spectrum activity.

The findings revealed that the Hep-2 cell strain was more susceptible to the toxic effects of the extracts compared to the AMN-3 cell strain, highlighting differences in genetic and molecular characteristics that influence each strain's reaction to plant compounds (Almughamisi et al, 2026). These variations might be due to differences in cell division rates, the efficiency of DNA repair systems, and the expression levels of apoptotic regulatory proteins like Bax, Bcl-2, and Caspase-3, as well as disparities in mitochondrial metabolic activity (Deniz-González et al, 2025). Consequently, Hep-2 cells may be more vulnerable to energy production inhibition and oxidative stress caused by compounds from the soursop plant (*Annona muricata*) (Rekha et al., 2024; Aljabali et al., 2025).

These results are consistent with numerous studies demonstrating that components of soursop (*Annona muricata*) possess strong antioxidant and anticancer properties, and that the level of these properties is affected by the extraction method, the plant part used, and the type of cell targeted (Deniz-González et al., 2025). Other studies have shown that the active compounds isolated from the leaves and seed of the plant are capable of inducing cytotoxicity, stimulating programmed cell death and necrosis, stopping the cell cycle, inhibiting the proliferation of cancer cells, as well as reducing the ability of cells to invade and spread. This makes the plant a promising source for developing anti-cancer therapeutic compounds (Liaw et al., 2002; Rosdi et al., 2015; Kuete et al., 2016).

5. CONCLUSION

This study showed that aqueous extracts of the peel and seeds of the soursop fruit (*Annona muricata*) are highly effective in protecting cells from the toxic effects of cancer drugs, by reducing chromosomal abnormalities and micronuclei, and increasing Mitotic index. The results also showed that both extracts possess concentration- and duration-dependent anti-cancer activity, significantly inhibiting the growth and survival of cancer cells. These results indicate that the peels and seeds of the soursop fruit, often overlooked agricultural by-products, represent a promising source of bioactive compounds with therapeutic potential, which could be used to develop natural value-added products for the pharmaceutical and healthcare sectors.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Scientific Research Authority, Iraq, for its valuable support of this research. Appreciation is also extended to the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq, for providing the facilities and technical support necessary to complete this study.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization, Methodology, Investigation, Laboratory Analysis, Data Curation, Formal Analysis, Visualization, and Writing—Original Draft.

Author 2: Review & Editing and Supervision.

All authors approved the final version of the manuscript. .

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The experimental procedures involving animals were conducted in accordance with the applicable guidelines for the care and use of laboratory animals.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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