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Potential Protective Effect of Bromelain and Pineapple Extract on Gamma Irradiated Female Rats: A Study of Oxidative Stress, Apoptosis, and Hormonal Changes

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Abstract In the study, the pure bromelain and its natural source in pineapple extracts were examined for their protective effects on ovary function in Albino rats exposed to gamma radiation. Female Albino rats (n = 90, prepubertal, 90-140 g each) were equally divided into six experimental groups: G1 was provided with distilled water, G2 exposed to a single dose of gamma radiation (8.3 Gy), G3 was orally administered with 7 mg/kg body weight bromelain for a week, G4 resemble G3 followed by exposure to irradiation, G5 was orally administered 30 mg/kg body weight *Ananas comosus* for a week, and G6 resembled G5 followed by exposure to irradiation. Rats were sacrificed one-hour, 24h, and 4 days post irradiation and histopathological alterations, granulosa cell viability and oxidative stress biomarkers and apoptosis were evaluated in the ovaries. The irradiated group experienced increased FSH and MDA levels with a reduction in E2 levels and enhanced *CAT* and *SOD* expression with marked increase in apoptosis. Treatment with both *A. comosus* extract and bromelain alleviated the adverse effects induced by the radiation.

Keywords: *pineapple extract, bromelain, oxidative stress, hormonal changes, gamma radiation, ovaries*

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1. Introduction

Organisms are naturally exposed to various forms of ionizing radiation throughout their lifetime. In particular, alpha particles have the highest ionizing power but lowest ability to penetrate living tissue, beta particles are less ionizing but slightly more penetrating, while gamma rays can pass through an individual and cause tissue damage [1,2], and x-rays, the only man-made version, are most commonly used in medical imaging [2,3]. Studies have reported that each type of these radiations can cause DNA mutations and chromosomal aberrations [1,4], though gamma and x-rays are particularly more harmful as they generate reactive oxygen species (ROS) [5,6,7,8] and engender cellular oxidative stress [9,10], leading to numerous chronic conditions such as cancer, asthma, arthritis, and cardiovascular disease [11].

Radiation therapy (RT) is one of the most effective treatments for cancer [5,12] by triggering apoptosis (programmed cell death) and necrosis (accidental cell death), respectively, through breaking chemical bonds in the DNA backbones of mutant cells [6] and stimulating lymphocytes and macrophages to secrete inflammatory cytokines such as interleukin-1, interleukin-2, and tumor necrosis factor-alpha [4]. For ovarian cancer patients, notably, studies showed that RT promotes apoptosis in follicular cells by activating the cytochrome c and caspase pathway [7]. Even though this treatment is considered effective, it is also known to heighten the risk of premature menopause and infertility [13]. Though these effects depend on the patient's age, site administered, fractionation schedule, and amount and type given. In general, the median lethal dose of RT resulting in permanent oocyte loss is less than 2 Gy; however, younger individuals may be more resistant than the elderly to this consequence [14].

Given the aforementioned findings, it is important to understand the anatomy and the functionality of the ovary. In brief, this endocrine gland consists of follicles that are composed of an outer layer of theca cells and an inner layer of granulosa cells (GCs), which surround and aid the oocyte-cumulus cell complex [15,16]. Development of the follicles is characterized by the prenatal and antral phases, wherein follicles undergo changes in size, morphology, and physiology while progressing from the primordial to preovulatory stage. One of the markers of primary ovarian insufficiency in the age of 40 are increases in serum levels of gonadotropin, follicle-stimulating hormone (FSH), and

luteinizing hormone (LH) [13,17]. These conditions can give rise to menopausal symptoms like hot flashes, fatigue, vaginal dryness, decreased libido, and atrophy of the breast at any age, though if occurring before menarche, it prevents the development of secondary sex characteristics. Following exposure to RT, pyknosis – condensation of chromatin in cells undergoing necrosis or apoptosis – can be seen in GCs within just a few hours. Evidence demonstrates that this ultimately leads to oocyte loss, follicular atrophies, depressed fertilization, and poor embryo quality and implantation rates [13,17]. In the medical felid about 70-80% of the gamma and X-ray effects are indirectly by generation of free radicals which induced oxidative stress and lead to many diseases [5].

Recently, owing to the deleterious effects of RT as evidenced above, there has been growing interest in the use of plants for protection against ROS [18,19]. Pineapple (*Ananas comosus*), noteworthily, is high in vitamins (i.e., A, B1, B2, B3, B4, and C), minerals (i.e., calcium, phosphorus, iron, copper, zinc, manganese, and potassium), and polyphenols which assist in the production of reproductive hormones and scavenge free radicals [10,18-23]. In addition, pineapple's core contains a high concentration of bromelain [24,25], which is a group of enzymes involved in the proteolysis of the cellular matrix and stimulation of monocytes for inhibiting cancer growth [24-28].

It has been reported that bromelain-fed mice, showed good insecticide detoxification markers as indicated by increased catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, and reduced glutathione (GSH) and decreased malondialdehyde (MDA) level [29]. In another study, treatment with pineapple peel extract for 28 days significantly reduced plasma MDA and CAT levels among subjects given alcohol [19].

The aim of the current study was to study potential protective effect of commercially available bromelain and its natural source extract in pineapple on the hormone levels, cell death, and GC viability of the ovaries of gamma irradiated rats.

2. Materials and Methods

2.1. Experimental Animals

Albino rats (n = 90, prepubertal, female, 90±10 g) were procured from King Saud University's animal house and maintained for a week under regular conditions (temperature (24 ± 2°C), humidity (40–70 %), and illumination (12/12-hour light/dark cycle). The rats were also given a conventional food and free access to water. All animals were handled according to the recommendation of the Ethics Committee at King Saud University (KSU), Riyadh, Saudi Arabia.

2.2. Irradiation

The animals were irradiated with a single dosage of 8.3 Gy using a Gamma cell 40 Extractor (Cesium 137) at the central lab of King Saud University. Each animal received whole-body exposure of 8.3 Gy, at a dose rate of 0.830 Gy/min, during the experimental period [7].

2.3. Preparation of Pineapple Juice

Pineapples were obtained from a local fruit market in Riyadh City, then cleaned and dried. They were chopped up and 650 g portions were blended and the concentrated extract at was stored at 4°C until further use. On the day of the experiment, the extract was diluted with nine parts distilled water [30].

2.4. Preparation of Bromelain

Bromelain was purchased from Sigma-Aldrich (St. Louis, MO, USA) and a dosage of 7 mg/kg body weight was prepared in distilled water [30].

2.5. Experimental Design

Prior to the experiment, all rats were weighed, assessed for growth, and inspected for diseases. The rats were placed into six groups, each with 15 rats. The details of each group are as follows:

Group 1, Rats administered distilled H_2O (Control group).

Group 2, Whole body gamma irradiated at a dose of 8.3 Gy, (0.830 Gy/min).

Group 3, Rats administered bromelain at a dosage of 7 mg/kg body weight by oral gavage for a week.

Group 4, Rats irradiated and pretreated with bromelain at a dosage of 7 mg/kg body weight by oral gavage for a week, then exposed to whole-body gamma radiation of 8.3 Gy, at a dose rate of 0.830 Gy/min.

Group 5, Rats were administered pineapple extract at a dose of 30 mg/kg body weight orally for a week.

Group 6, Rats pretreated with pineapple extract a dose of 30 mg/kg body weight orally for a week, then irradiated with 8.3 Gy, at a dose rate of 0.830 Gy/min.

All animals were housed with free access to water and rat chow. A total of five rats were sacrificed from each group at one hour, 24 hours, and four days post irradiation, following cessation of the treatment., Blood was immediately obtained from the trunk and collected in a tube, left to clot at room temperature, centrifuged for 15 minutes at 3000 rpm, and then the serum was aliquoted and stored at -80°C until further analyses. Ovaries were immediately excised, washed with ice-cold saline, and weighed. Samples were stored at -80ºC until further analysis.

2.6. Histopathological Examination

Samples of ovaries were fixed in 10% formalin overnight and then put ethanol (ranging from 70% to 100%). Next, the ovaries were placed in xylene, embedded in paraffin wax blocks, cut into 4–5-µ sections, placed on a heated water bath, and mounted onto glass slides. Dried samples were rehydrated in ethanol, stained with hematoxylin and eosin, and examined under a light microscope [7].

2.7. Determination of GC Viability

The Vi-CELL® Cell Viability Analyser (Beckman Coulter, USA) was utilized to determine the viability of GCs [31,32].

2.8. Assessment of Oxidative Stress

Lipid peroxidation was measured levels of MDA which react with thiobarbituric acid to yield a pink colored trimethine complex exhibiting at 532 nm. [33]. Activities of GSH, SOD, and CAT were determined using the MBS046356 commercial assay kit (BioSource, USA), 706002 commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA), and 707002 commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA), respectively.

2.9. Determination of Hormone Levels

Levels of serum estrogen (E2) and FSH were quantified using the CSB-E05110r enzyme-linked immunosorbent assay (Cusabio, USA) and CSB-E06869r enzyme-linked immunosorbent assay (Cusabio, USA), respectively.

2.10. Determination of Lactate Dehydrogenase (LDH) Levels

Serum LDH activity was determined from a coupled enzymatic reaction using the K311-400 commercial assay kit (BIOVISION, USA).

2.11. Quantification of Gene Expression and Cell Death

Ovarian tissues (≤ 30 mg) were lysed and homogenized in 360 µL lysis buffer. Next, DNA and RNA from the tissue samples were purified using the AllPrep DNA/RNA Mini Kit (#80204, Qiagen, Toronto, Ontario, Canada), and then their concentration and quality were assessed using a spectrophotometer (NanoDrop, Wilmington, DE, USA) at wavelengths of 260/280 nm.

From the RNA, 1 µg and random primers were used to synthesize cDNA via RevertAid H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Burlington, Ontario, Canada) according to the manufacturer's instructions. Following this, real-time polymerase chain reaction was performed in triplicates on all samples using Power SYBR Green (Life Technologies, Carlsbad, CA) and an Applied Biosystems 7500 Instrument (Foster City, CA). The cycling conditions was 95°C for 3 min, followed by 40 cycles at 95°C for 15 sec, 61°C for 20 sec, 72°C for 20 sec, 72°C for 2 min, and then termination at 4°C. The ΔCt was calculated by subtracting the Ct of β-actin from that of each sample using the Applied Biosystems StepOne software (Thermo Fisher Scientific, Waltham, MA). Next, the Pfaffl method was used to calculate relative gene expression. All primers were synthesized by Integrated DNA Technologies, Inc. (Illinois, USA); sets used for *CAT* were: (F) 5'-ATT GCC GTC CGA TTC TCC-3', (R)5'-CCA GTT ACC ATC TTC AGT GTA G-3', sets used for *SOD* were: (F) 5'-GAG CAG AAG GCA AGC GGT GAA-3', (R) 5'-CCA CAT TGC CCA GGT CTC-3', and sets used for *GPx* were: (F) 5'-AAC GTG GCC TCG CAA TGA-3', (R) 5'-GGG AAG GCC AGG ATT CGT AA-3'. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene because it is commonly employed in comparisons of gene expression data.

DNA is extracted from 30 mg from each tissue sample using Qiagen kit according to the manufacturing instructions. The integrity of the DNA was assessed by gel (1.5% agarose) electrophoresis containing ethidium bromide and visualized by UV light (300 nm) and photographed to assess DNA fragmentation as a marker of apoptosis. Caspase-3 (CPP32) activity, was analyzed using the K106-100 commercial assay kit (Biovision, USA), which relies on spectrophotometric detection of chromophore p-nitroaniline (pNA) at 400-405 nm following its cleavage from the labeled substrate, DEVD-pNA. The difference in light absorbance of pNA between apoptotic sample compared with uninduced control allows for the determination of the fold increase in CPP32 activity.

2.12. Statistical Analysis

Results are expressed as mean ± standard error (SE). One-way analysis of variance evaluated differences between multiple groups, and Duncan's test was performed when differences were significant. The statistical package program SPSS (ver. 23, Chicago, IL) was used to conduct data analyses. All p-values were two-sided and $p \leq 0.05$ was considered statistically significant.

3. Results

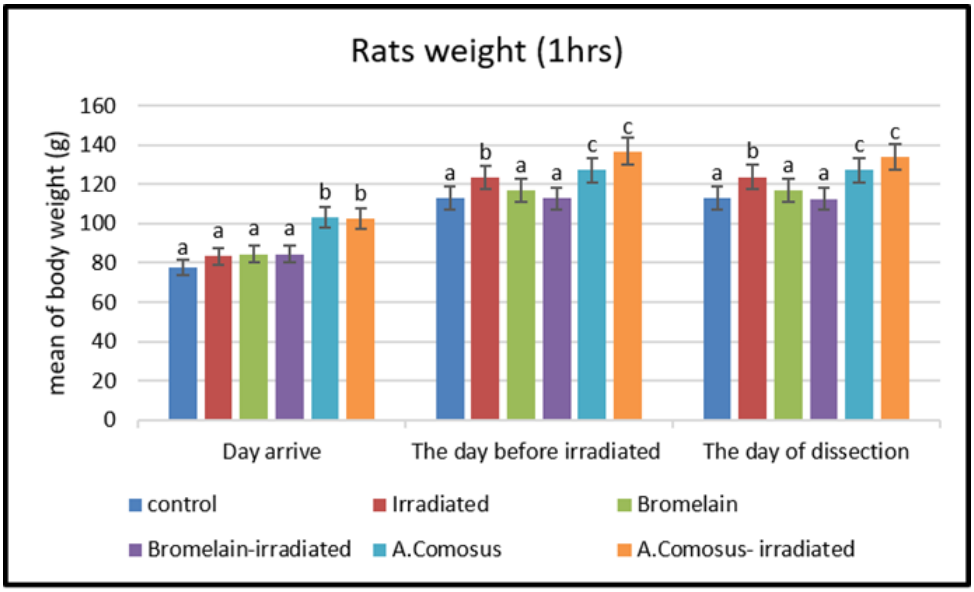
The aim of this research was to examine the abnormalities induced by gamma irradiation within the parameters for ovary functions in female rats compared by control groups. The gene expression of CAT, SOD and Gpx in ovary, was evaluated. In addition, biomarkers of oxidative stress, hormonal and cell death changes were performed. The proposed protective effect of *A. comosus* extract and bromelain were studied. The results showed that *A. comosus* extract and bromelain supplementation did not significantly effect on oxidative stress and hormonal level for ovary functions in the non-irradiated group throughout all post-treatment times, when compared to the control levels. Treatment with *A. comosus* or bromelain prior to irradiation protected DNA and alleviated the adverse effects induced by the radiation.

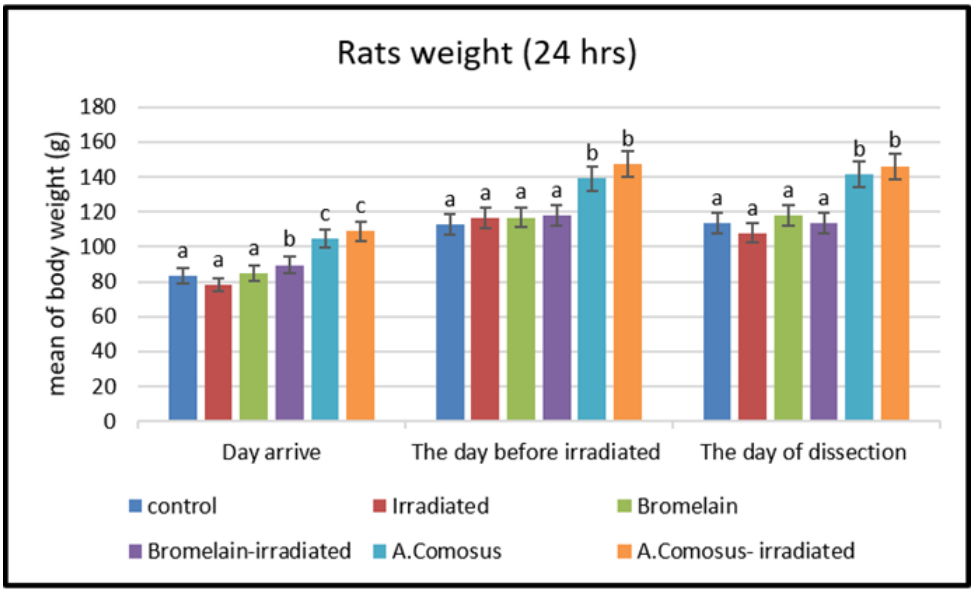
3.1. Body and Ovarian Weight

No significant change was observed in the mean body weight of rats in all the experimental groups between the day before the exposure to the radiation and the day of dissection (Figure 1 and Figure 2).

When compared to the control group, the weight of the right and left ovaries of the groups treated with bromelain or *A. comosus* extract increased significantly (values, $p \leq 0.05$ for all). By comparing bromelain and *A. comosus* of non-irradiated group there was no significant change between ovarian weight at all intervals. Moreover, a significant decrease was observed in the ovary weight of the irradiated group when compared to the control (values, $p \leq 0.05$).

There was a significant decrease in right ovarian weight at interval (1 h, 4 days) in bromelain – irradiated and *A. comosus* – irradiated group compared to irradiated rats.





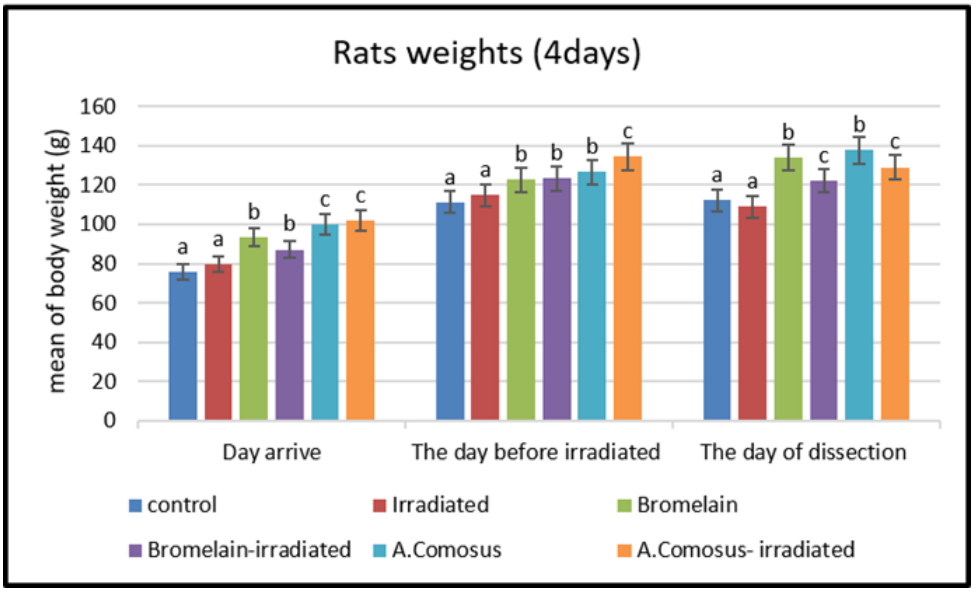


Figure 1. Mean body weight (g) during the experimental period at intervals of one hour, 24 hours, and 4 days. Superscripts indicate significant and non-significant differences

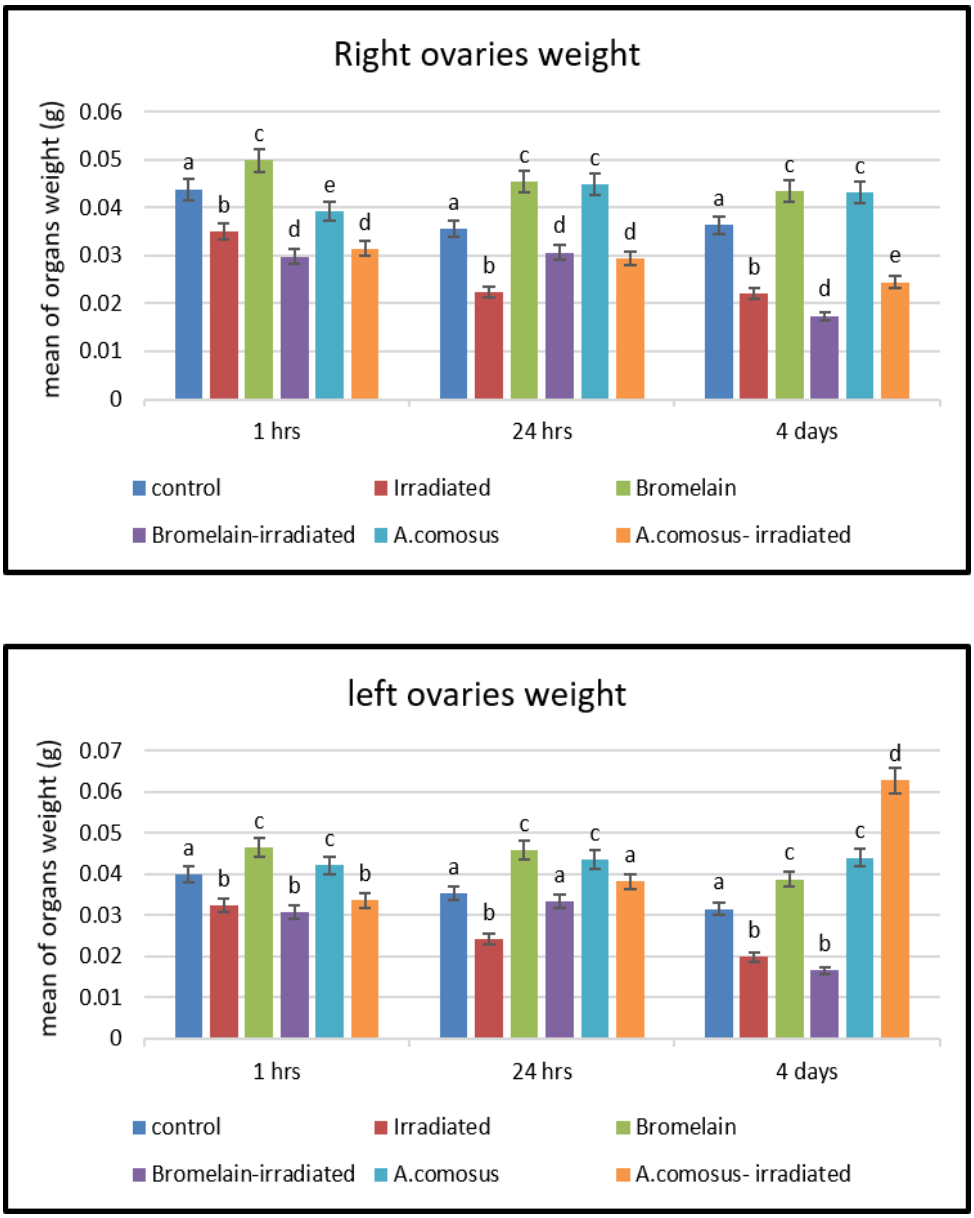


Figure 2. Mean weight (g) of the left and right ovaries during the experimental period at intervals of one hour, 24 hours, and 4 days. Superscripts indicate significant and non-significant differences

3.2. Histopathology of the Ovary

Ovarian sections from the control group are shown in Figure 3A, C, and E. Specifically, the cortex is a peripheral zone containing follicles and a stroma with connective tissue, and the medulla is located in the center of the ovary and consists of connective tissue with blood vessels.

There were no histological alterations in the ovaries of irradiated rats at one hour, although hemorrhage in the stroma and increased oocyte degeneration with follicular atresia were apparent at day 4. Bromelain and *A. comosus* extract were determined to be beneficial in protecting the structural integrity of the ovaries before irradiation. (Figure 3B, D, and F).

3.3. GC Viability

No significant difference was observed in the GC viability between groups treated with bromelain and *A. comosus* vs. the control group at all-time intervals. However, a highly significant decrease was observed in GC viability of the irradiated group relative to that of the control group at all intervals (50.4 ± 0.3 vs. 65.2 ± 3.2, 45.56 ± 0.1 vs. 70.98 ± 0.2, and 49.36 ± 0.4 vs. 73.1 ± 0.1, respectively, $p \leq 0.05$, 0.01 and 0.01, respectively). On comparing the data obtained from treated – irradiated rats and irradiated rats, Bromelain and *A. comosus* extract were found to have a significant protective effect against radiation-induced damage in the granulosa cell of the ovarian tissue. (Table 1).

3.4. Biochemical Assays

3.4.1. MDA and GSH Levels

MDA levels were found to be significantly lower. at all intervals compared to the control group in groups treated with bromelain or *A. comosus* (values, $p \leq 0.05$ for all). MDA levels in the irradiated groups were significantly higher than in the control group. at all intervals (45.52 ± 0.2 vs. 25.30 ± 0.0, 48.13 ± 5.9 vs. 24.40 ± 0.2, and 40.67

± 1.4 vs. 26.52 ± 4.0, respectively, p ≤0.01,0.001 and 0.001, respectively), although irradiated groups treated with bromelain or *A. comosus* displayed significant reduction in MDA levels relative to the irradiated group at all intervals.

When compared to the control group, the irradiated group had significantly lower GSH levels at all intervals. (50.99 ± 0.9 vs. 60.00 ± 2.0, 49.23 ± 0.7 vs. 64.15 ± 1.7, and 52.46 ± 0.6 vs. 64.80 ± 0.2, respectively, p ≤0.05,0.01 and 0.01, respectively). On comparing the data obtained in irradiated rats with treated irradiated rats, it was observed that bromelain and *A. comosus* significantly reversed the reduction in ovarian GSH level induced by the exposure to radiation (Table 2).

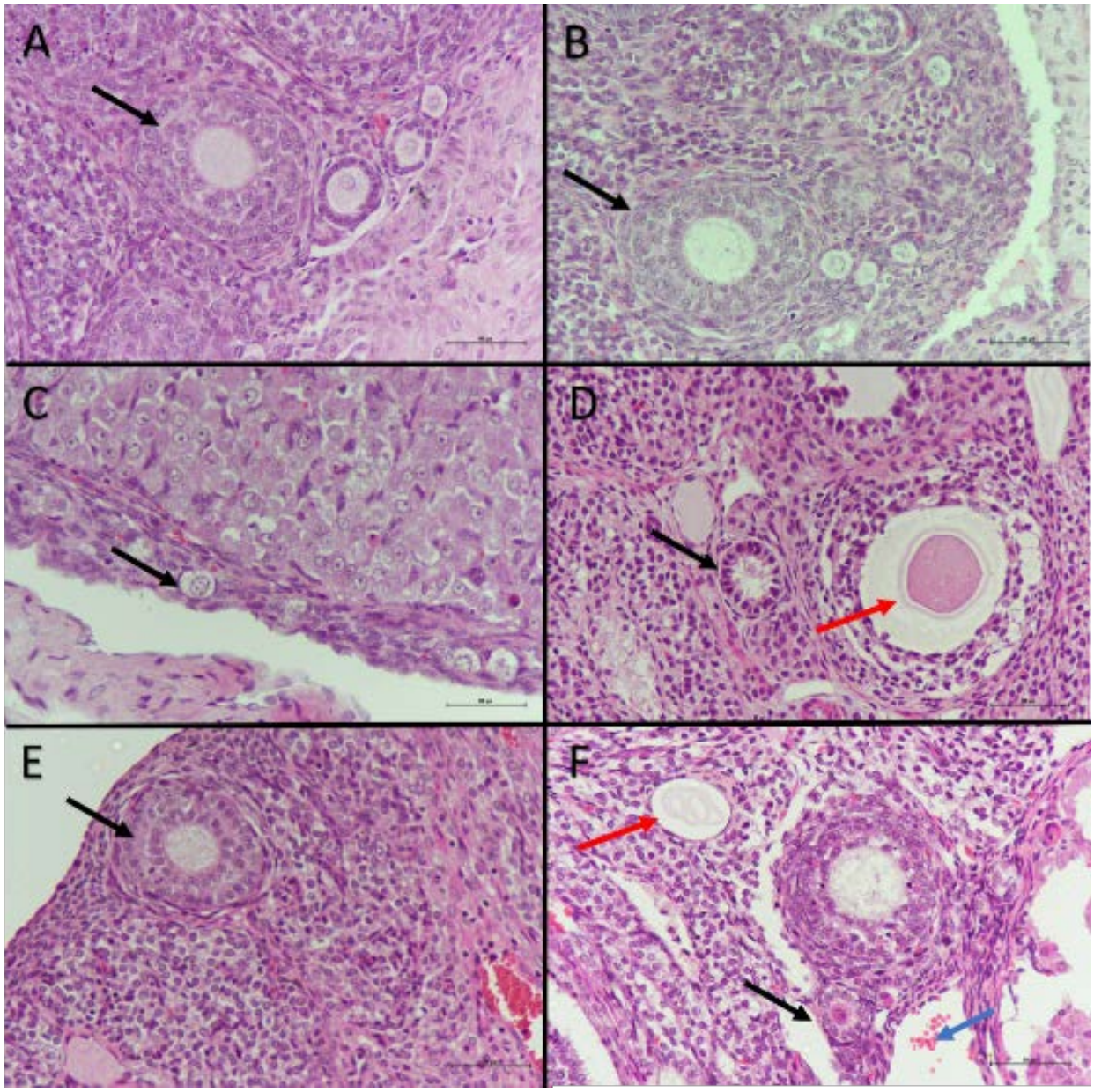


Figure 3. Photomicrographs of hematoxylin and eosin-stained ovarian sections at 4 days (magnification of 40X). Histological sections from the control group (A), bromelain-treated group (C), and *A. comosus*-treated group (E) show a similarity in organization but differences in follicular growth (black arrows). Histological sections from the irradiated group (B) show degeneration of oocytes in primary follicles (black arrows), irradiated group treated with bromelain (D) and irradiated group treated with *A. comosus* (F), have normal follicular growth (black arrows), oocytes in primary follicles are degenerating (red arrows) and little hemorrhage (blue arrows)

Table 1. Mean ± SE percentages of granulosa cell viability in experimental groups. Superscripts indicate significant and non-significant differences

Groups / Times	Group 1 Control	Group 2 Irradiated	Group 3 Bromelain	Group 4 Bromelain- irradiated	Group 5 *A.comosus*	Group 6 *A.comosus*-irradiated
Granulosa cell viability (1hour)	65.2±3.2 [a]	50.4±0.3 [b]	72.12±0.3 [a]	72.12±0.3 [a]	74.44±1.8 [a]	76.20±1.1 [a]
Granulosa cell viability (24hours)	70.98±0.2 [a]	45.56±0.1 [b]	67.49±0.1 [a]	69.3±0.4 [a]	71.38±0.2 [a]	69.32±0.5 [a]
Granulosa cell viability (4days)	73.1±0.1 [a]	49.36±0.4 [b]	74.6±0.6 [a]	75.4±1.4 [a]	71.10±0.2 [a]	72.8±0.1 [a]

Table 2. Mean ± SE changes in the levels of MDA (nmols/g fresh tissue) and reduced glutathione (GSH) (mmol/L) in ovaries during the experimental period. Superscripts indicate significant and non-significant differences

Groups / Times	Group 1 Control	Group 2 Irradiated	Group 3 Bromelain	Group 4 Bromelain-irradiated	Group 5 *A.comosus*	Group 6 *A.comosus*-irradiated
MDA (1hr)	25.30±0.0 [a]	45.52±0.2 [b]	20.85±1.7 [c]	30.69±0.9 [d]	23.73±0.1 [e]	28.68±0.3 [d]
MDA (24 hrs)	24.40±0.2 [a]	48.13±5.9 [b]	22.43±6.2 [c]	34.57±2.7 [d]	21.7±3.1 [a]	35.4±3.9 [d]
MDA (4 days)	26.52±4.0 [a]	40.67±1.4 [b]	21.04±2.7 [c]	30.16±3.4 [b]	24.52±3.5 [c]	27.26±2.8 [a]
GSH (1 hr)	60.00±2.0 [a]	50.99±0.9 [b]	70.57±0.0 [c]	50.62±0.8 [b]	65±0.9 [c]	55.57±0.0 [b]
GSH (24 hrs)	64.15±1.7 [a]	49.23±0.7 [b]	70.23±0.7 [c]	60.24±1.1 [a]	63.34±0.7 [a]	50.33±0.7 [b]
GSH (4 days)	64.80±0.2 [a]	52.46±0.6 [b]	75.48±1.0 [c]	65.27±1.6 [a]	67.17±3.9 [a]	62.74±0.8 [a]

3.4.2. Activity of Antioxidant Enzymes

The bromelain-treated group showed a substantial increase in SOD activity at all periods. (2.410 ± 0.2 vs 1.410 ± 0.0, 2.120 ± 0.1 vs. 1.620 ± 0.8, and 1.750 ± 0.2 vs. 1.500 ± 0.3, respectively, $p \leq 0.05$ for all), In addition, when compared to the control group, there was a significant drop in CAT activity after 24 hours. (values, $p \leq 0.05$).

The group treated with *A. comosus* demonstrated no significant change in SOD activity at one hour but did show an increase in SOD activity on day 4 compared to the control group (2.160 ± 0.4 vs. 1.500 ± 0.3, $p \leq 0.05$). Furthermore, this group had a large rise in CAT activity at 24 hours and a significant drop on day 4 compared to control. (values, $p \leq 0.01$ and $p \leq 0.05$ respectively).

At 24 hours and day 4, the irradiated group had a considerable increase in SOD activity. (2.470 ± 0.2 vs. 1.620 ± 0.8 and 2.830 ± 0.4 vs. 1.500±0.3, $p \leq 0.05$ for all). Also, a significant increase in CAT activity at 24 hours and day 4 was seen in this group compared to the control group (51.00 ± 0.2 vs. 41.44 ± 0.6 and 71.91 ± 0.5 vs. 54.78 ± 0.3, respectively, $p \leq 0.05$ for all).

Interestingly, when compared to the irradiated group, the bromelain-treated group demonstrated a substantial decrease in SOD activity at 24 hours and day 4. (0.660 ± 0.3 vs. 2.470 ± 0.2 and 0.820 ± 0.2 vs. 2.830 ± 0.4, respectively, $p \leq 0.01$ for all), and the irradiated group treated with *A. comosus* experienced a significant decrease in SOD activity at 24 hours and day 4 compared to the irradiated group (values, $p \leq 0.05$ for all). In addition, there was a significant decrease in CAT activity on day 4 in irradiated groups treated with bromelain or *A. comosus* compared to that of the irradiated group (values, $p \leq 0.01$ for all) (Table 3).

3.4.3. Hormone Levels

The bromelain group showed a significant increase in FSH levels at all intervals compared to the control group (12.94 ± 0.4 vs. 9.84 ± 1.1, 13.90 ± 0.24 vs. 11.64 ± 0.1, and 13.59 ± 0.71 vs. 11.26 ± 1.0, respectively, $p \leq 0.5$ for all), as well as did the group treated with *A. comosus* extract compared to the control group (13.89 ± 2.8 vs. 9.84 ± 1.1, 14.93 ± 0.04 vs. 11.64 ± 0.1, and 14.85 ± 0.5 vs. 11.26 ± 1.0, respectively, $p \leq 0.05$ for all).

The bromelain group displayed a non-significant decrease in E2 levels at all intervals compared to the control group (166.8 ± 1.1 vs. 205.89 ± 2.7, 185.2 ± 11.1 vs. 268.80 ± 5.4, and 164.00 ± 14.20 vs. 186.60 ± 2.0, respectively, $p \leq 0.05$ for all), as did the group treated with *A. comosus* compared to the control group (198.89 ± 3.7 vs. 205.89 ± 2.7, 260.00 ± 4.52 vs. 268.80 ± 5.4, and 174.40 ± 12.9 vs. 186.6 ± 2.0, respectively, $p \leq 0.05$ for all).

The exposure to radiation showed an increase in FSH levels at all intervals compared to the control group (16.62 ± 3.4 vs. 9.84 ± 1.1, 17.36 ± 0.1 vs. 11.64 ± 0.1, and 18.71 ± 0.8 vs. 11.26 ± 1.0, respectively, $p \leq 0.001$ for all), whereas At all intervals, E2 levels in this group were considerably lower than in the control group. (80.44 ± 1.2 vs. 205.89 ± 2.7, 79.7 ± 12.8 vs. 268.80 ± 5.4, and 69.3 ± 8.7 vs. 268.80 ± 5.4, respectively, $p \leq 0.001$ for all).

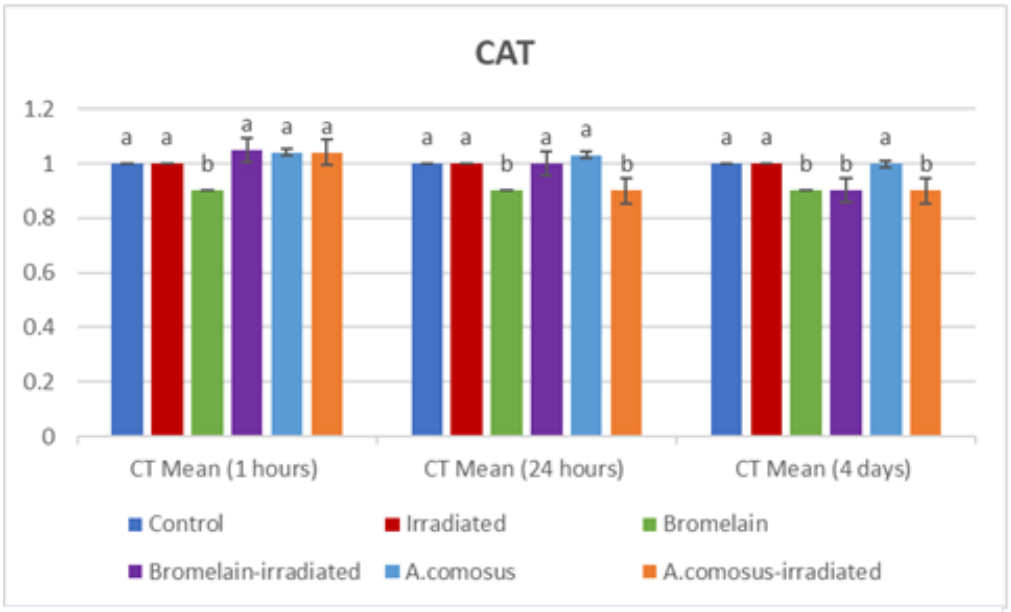
The irradiated groups treated with bromelain or *A. comosus* extract demonstrated a decrease in FSH levels at all intervals compared to the irradiated group (values, $p \leq 0.05$ for all). Moreover, there was a considerable increase in E2 levels at all intervals for the group exposed to radiation and treated with bromelain or *A. comosus* compared to the irradiated group (values, $p \leq 0.05$ for all) (Table 4).

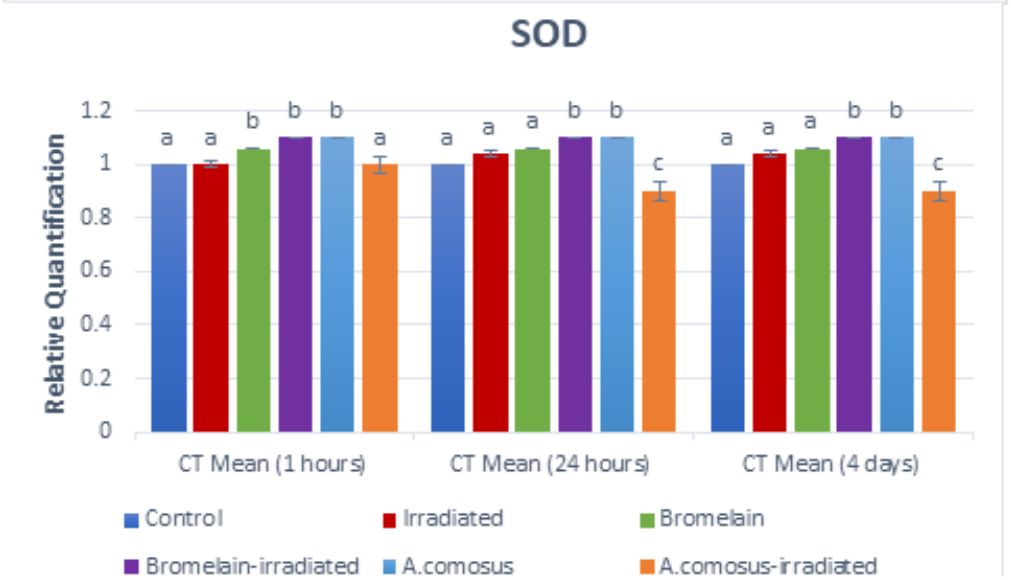
Table 3. Mean ± SE changes in the activities of superoxide dismutase (SOD) (U/mL) and catalase (CAT) (U/mmol/L) in ovaries during the experimental period. Superscripts indicate significant and non-significant differences.

Groups / Times	Group 1 Control	Group 2 Irradiated	Group 3 Bromelain	Group 4 Bromelain-irradiated	Group 5 *A.comosus*	Group 6 *A.comosus*- irradiated
CAT (1hours)	78.99±0.3 [a]	77.18±0.3 [a]	83.61±0.3 [a]	65.91±0.7 [b]	71.16±0.3 [b]	29.85±0.1 [c]
CAT (24hours)	41.44±0.6 [a]	51.00±0.2 [b]	28.00±0.3 [c]	62.63±0.2 [d]	71.52±0.6 [e]	55.22±0.4 [b]
CAT (4 days)	54.78±0.3 [a]	71.91±0.5 [b]	64.25±0.3 [c]	60.14±0.2 [c]	49.37±0.2 [a]	58.72±0.4 [c]
SOD (1hours)	1.410±0.0 [a]	1.200±0.0 [b]	2.410±0.2 [c]	1.460±0.1 [a]	1.410±0.0 [a]	1.220±0.0 [b]
SOD (24hours)	1.620±0.8 [a]	2.470±0.2 [b]	2.120±0.1 [c]	0.660±0.3 [d]	1.280±0.2 [e]	1.040±0.3 [f]
SOD (4days)	1.500±0.3 [a]	2.830±0.4 [b]	1.750±0.2 [c]	0.820±0.2 [d]	2.160±0.4 [e]	1.350±0.2 [a]

Table 4. Mean ± SE changes in serum levels of follicle-stimulating hormone (FSH) (U/mL) and estrogen (E2) (pg/mL) during the experimental period. Superscripts indicate significant and non-significant differences

Groups / Times	Group 1 control	Group 2 Irradiated	Group 3 Bromelain	Group 4 Bromelain-irradiated	Group 5 *A.comosus*	Group 6 *A.comosus*- irradiated
FSH (1hr)	9.84±1.1 [a]	16.62±3.4 [b]	12.94±0.4 [c]	13.13±2.1 [c]	13.89±2.8 [c]	10.08±0.2 [a]
FSH (24hrs)	11.64±0.1 [a]	17.36±0.1 [b]	13.90±0.24 [c]	15.18±0.25 [c]	14.93±0.04 [c]	16.90±0.2 [c]
FSH (4 days)	11.26±1.0 [a]	18.71±0.8 [b]	13.59±0.71 [c]	15.30±0.01 [c]	14.85±0.5 [c]	15.84±0.7 [c]
Estradiol (1hrs)	205.89±2.7 [a]	80.44±1.2 [b]	166.8±1.1 [a]	90.2±4.1 [c]	198±3.7 [a]	97.4±0.8 [c]
Estradiol (24hrs)	268.80±5.4 [a]	79.7±12.8 [b]	185.2±11.1 [a]	89.5±7.8 [c]	260±4.52 [a]	90.7±35.6 [c]
Estradiol (4days)	186.6±2.0 [a]	69.3±8.7 [b]	164±14.20 [a]	100.8±1.99 [c]	174.4±12.9 [a]	95.2±3.9 [c]





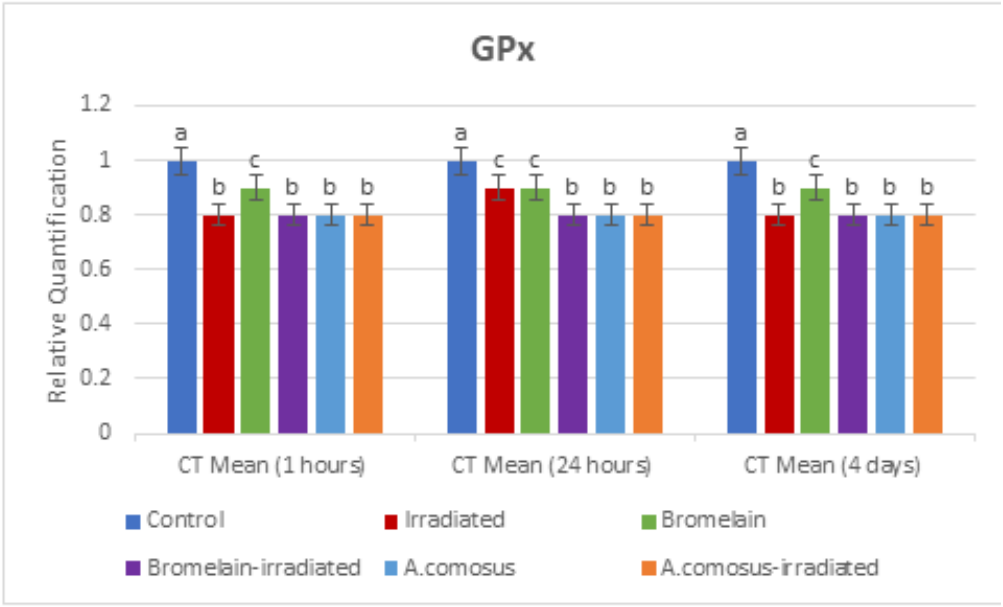


Figure 4. Effect of gamma irradiation on expression of catalase (*CAT*) (A), superoxide dismutase (*SOD*) (B), and glutathione peroxidase (*GPx*) (C) in rat ovaries. Real-time polymerase chain reaction was normalized to the quantity of glyceraldehyde-3-phosphate dehydrogenase mRNA. Superscripts indicate significant and non-significant differences

3.5. Molecular Analyses

3.5.1. Expression of *CAT, SOD,* and *GPx*

The gene expression of CAT, SOD, and GPx in the ovary was evaluated using real-time PCR. The gene expression of GAPDH was used as the endogenous control to normalize the expression level of the different genes. CAT and GPx expression were significantly lower in the bromelain-treated group.at all intervals compared to control (values, $p \leq 0.05$ for all), while, as compared to the control group, the group treated with *A. comosus* extract showed an increase in SOD expression and a decrease in GPx expression at all intervals.

There was no discernible difference between the irradiated and non-irradiated groups.in *CAT* and *SOD* expression at all intervals, but did show a significant decrease in *GPx* expression at all intervals when compared to the control group (values, $p \leq 0.05$ for all).

At day 4, the irradiated group treated with bromelain had considerably lower CAT expression than the irradiated group. (values, $p \leq 0.01$) However, when compared to the irradiated group, the SOD expression increased at all intervals. (values, $p \leq 0.05$ for all). The irradiated group treated with *A. comosus* extract showed a significant decrease in *CAT* and *SOD* expression at 24 hours and day 4 compared to the irradiated group (values, $p \leq 0.05$ for all) with a decrease in GPx expression only at 24 hours (values, $p \leq 0.05$ for all) (Figure 4).

3.5.2. Cell Death Markers

3.5.2.1. Serum Levels of LDH

When comparing groups treated with bromelain or *A. comosus* extract to the control group, no significant change in LDH levels was detected at any period. However, the irradiated group had a significant rise in LDH levels at all intervals compared to control. (195.6 ± 9.06 vs. 47.8 ± 17.4, 412.9 ± 55.4 vs. 45.9 ± 16.9, and 217.04 ± 11.1 vs. 29.73 ± 8.36, respectively, $p \leq 0.05,0.001,0.01$, respectively). On comparing the information gathered in the irradiated rats to that of treated irradiated rats, it is observed that bromelain or *A. comosus* extract treatment was efficacious against the radiation-induced necrosis compared to the irradiated group (Table 5).

3.5.2.2. DNA Fragmentation

The irradiated group exhibited DNA fragmentation at all intervals when compared with the control group, the bromelain irradiated-treated group had less DNA fragmentation at all intervals when compared to the irradiated group. Whereas, there was no DNA fragmentation detected at any time interval in the irradiated group treated with *A. comosus*, which indicated *A. comosus* had a marked protective effect against radiation-induced apoptosis (Figure 5).

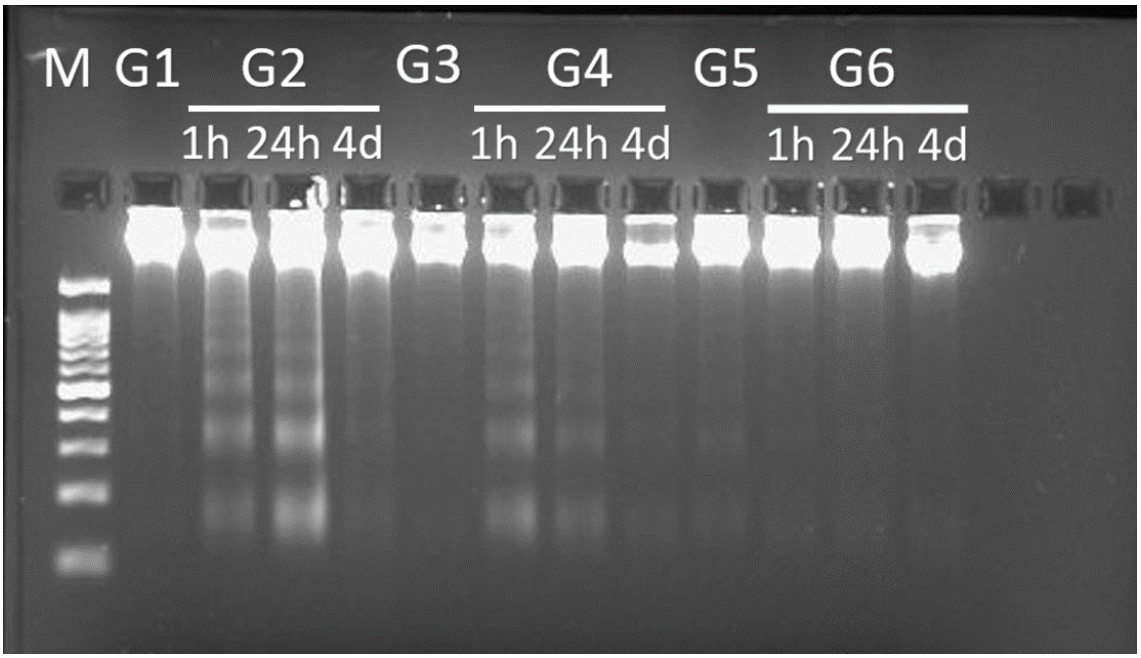


Figure 5. Photographs of DNA fragmentation in agarose gels from the control group (G1), irradiated group (G2), bromelain-treated group (G3), irradiated group treated with bromelain (G4), *A. comosus*-treated group (G5), and irradiated group treated with *A. comosus* (G6)

Table 5. Mean ± SE changes in the activity of serum lactate dehydrogenase (LDH) (Ul/L) during the experimental period. Superscripts indicate significant and non-significant differences

Groups / Times	Group 1 Control	Group 2 Irradiated	Group 3 Bromelain	Group 4 Bromelain-irradiated	Group 5 *A.comosus*	Group 6 *A.comosus*-irradiated
LDH (1hours)	47.8±17.4 [a]	195.6±9.06 [b]	48.2±3.60 [a]	168.2±12.0 [b]	25.72±3.6 [a]	194.7±1.1 [b]
LDH (24hours)	45.9±16.9 [a]	412.9±55.4 [b]	50.4±71.4 [a]	244.80±14.0 [c]	28.39±5.5 [a]	53.50±5.7 [a]
LDH (4days)	29.73±8.36 [a]	217.0±11.1 [b]	34.70±5.90 [a]	133.3±40.9 [c]	23.3±2.0 [a]	69.30±1.7 [d]

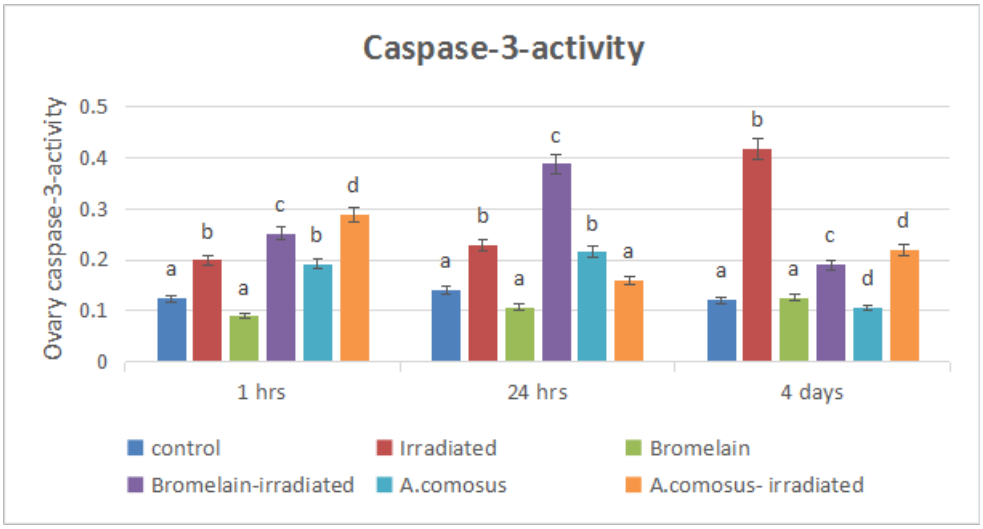


Figure 6. Mean ± SE changes in caspase-3 activity (ng/g tissue) in ovaries during the experimental period. Superscripts indicate significant and non-significant differences

3.5.2.3. CPP32 Activity

The irradiated group experienced an increase in CPP32 activity at all intervals compared to the control group (values, $p \leq 0.01,0.01,0.001$, respectively) (Figure 6). The treatment with bromelain and *A. comosus* extract before Radiation exposure resulted in a decrease in the level of CCP32 activity which mirrored the DNA fragmentation test results.

4. Discussion

Radiation can change the characteristics of the cell nucleus and cytoplasm, as mammalian germ cells are very sensitive to ionizing radiation. As one of the most prevalent cancer treatments, radiotherapy contributes to female infertility by causing premature ovarian failure (POF). Through the generation of free radicals, ionizing radiation causes oxidative damage to vital biological systems and biomolecules, including DNA, lipids, proteins, and membranes. [34].

The increased ROS production by granulosa cells appears to affect oocyte fertilization, embryo quality, and implantation rates. Furthermore, it seems that germ cells are more susceptible to oxidative stress than somatic cells [35,36].

Several antioxidants have been efficaciously used to ameliorate Radiation damage to the ovaries, such as resveratrol, melatonin, Shilajit and selenium Possibly by reducing oxidative stress [7].

A study was conducted to evaluate the impact of the pineapple extract and bromelain on hormone levels, histopathology and granulosa cell viability in ovaries of rats exposed to gamma radiation. In addition, in a radiation-induced ovarian failure experimental model, pineapple extract and bromelain were tested for their molecular radioprotective effects on different markers of oxidative stress, cell death, and gene expression (CAT, SOD, and GPx).

Cross sections of the ovary from the control group revealed primary, secondary, and antral follicles, whereas the irradiated group had degeneration of the GC layer, hemorrhage in the cortex layer, and fewer follicles. A close examination of ovarian cross sections, showed the presence of primary, secondary, and antral follicles in the control group, whereas irradiated rats displayed degeneration of the GC layer, hemorrhage in the cortex layer, and a lower number of growing follicles. However, administration of pineapple extracts prior to irradiation helped to maintain the overall integrity of the ovary, with reduced apoptosis, in specific, being detected at day 4. Results from previous studies agree with these findings [12,17,37]. irradiated rats had dramatically reduced viability of the granulosa cells, which may be attributable to an increase in p53, a process that occurs through post-translational mechanism and the regulation of expression of genes involved in cell cycle progression. According to the present study, pre-treatment of irradiated animals with bromelain or *A. comosus* markedly increased the GC viability [38,39].

The interaction of free radicals with polyunsaturated fatty acids contained in cellular membranes caused a rise in MDA levels (i.e., lipid peroxidation) in irradiated rats. [40] as well as a considerable drop in the amount of GSH. The reduction in GSH levels after exposure to radiation could be due to GSH reacting with free radicals to form thiol radicals, which are linked to the production of oxidized glutathione. [41]. The current study's findings revealed that the the ingestion of pineapple extract helped to maintain GSH levels in the irradiated treated rats relative to irradiated rats, which is in consensus with the results reported by Bilbao et al. [42].

In this investigation, irradiated rats treated with bromelain or *A. comosus* extract had less lipid peroxidation, when compared to the irradiated group, suggesting that pineapple extract can scavenge free radicals and thereby provide a potent ameliorative effect against radiation induced oxidative stress. In a prior investigation, the antioxidant action of ghrelin was found to be responsible for a significant reduction in lipid peroxidation. [43].

In terms of the of the oxidative stress implicated in follicular atresia, the study assessed GPx, SOD and CAT – antioxidant enzymes expressions that combat ROS, catalyze the dismutation of superoxide radicals and reduction of hydrogen peroxide, respectively [8,10,29,44].

Moreover, the irradiated group showed no remarkable change in *SOD* and *CAT* expression compared to the control group, although it did demonstrate a clear decrease in *GPx* expression. The decreased GPx expression in the ovary, may be one of the major factors that is responsible for follicular regression [45].

Treatment with bromelain was found to lower expression of *CAT* and *GPx* but not that of *SOD* when compared to the control group, whereas treatment with *A. comosus* significantly increased *SOD* expression and decreased *GPx* expression when compared to the control group. Administration of *A. comosus* before irradiation decreased gene expression of all the antioxidant enzymes; however, ingestion of bromelain prior to irradiation elevated *CAT* and *SOD* expression and decreased *GPx* expression. Changes in MDA, GSH levels, and CAT, SOD, and GPx expression among rats in this study could indicate that pineapple extract has a protective impact on the female reproductive system.

FSH is critical for maintaining ovarian function [10], and evidence reveals its levels can be compromised after whole-body irradiation or RT [12]. FSH levels were found to rise after irradiation, which could be due to aberrant steroid release from the ovaries. Administration of pineapple extract prior to this treatment was successful at preventing FSH disruptions. Additionally, levels of E2, a hormone released from GCs upon stimulation by FSH, were lowered as a result of irradiationand this is likely due to adverse effects of the radiation on GCs. These findings reflect the presence of typical ovarian failure [46].

The oxidative stress caused by radiation was dramatically reduced when rats were pre-treated with pineapple extracts. These findings confirm pineapple extract's powerful antioxidant action in avoiding lipid peroxidation and preserving tissue and cell integrity and function, which promoted follicular growth by lowering ROS levels and enhancing GPx expression [17]. Thus, the mechanisms that have been suggested are stimulating the granulosa cell proliferation and improving follicular development through enhanced GPx expression, and reduction in oxidative stress indicators. Also, radiation induces apoptosis by ROS production [47]. ROS caused mitochondrial membrane disruption, which resulted in the release of cytochrome c into the cytoplasm, activating caspases and triggering apoptosis [48].

Ovarian granulosa cells are important regulators of ovarian physiology, such as ovulation and luteal regression, which are important for fertility and conception. [49].

The ovarian follicles do not respond to high levels of FSH induced by irradiation and do not secrete E2 in ovarian failure. Subsequently, FSH in this case stimulates apoptosis in ovarian follicles [50]. In fact, granulosa cell death appears to be the cause of follicular atresia. [51].

When apoptotic markers were measured in ovarian granulosa, it was discovered that radiation significantly increased DNA fragmentation and caspase 3 activity when compared to a control group. Radiation activates the apoptotic process by inducing oxidative stress or ionization, which disrupts mitochondrial membrane integrity and releases cytochrome c from the inner mitochondrial membrane into the cytosol, prompting apoptosis via caspase activation.

It has been reported that cleavage of chromosomal DNA into fragments of oligonucleosomal size is a biochemical hallmark of apoptosis and can occur after brain injuries, bacterial infections, and treatment with radiation [52]. In this research, the irradiated group had more DNA fragmentation than the irradiated group treated with *A. comosus*, which showed no fragmentation at all. In addition, ionizing radiation-induced apoptosis could be inhibited by pineapple extracts through lowering caspase 3 activity.

Thus, another way pineapple extracts aided follicular growth is by lowering oxidative stress-induced apoptosis. Ovarian radio-protection by pineapple extracts may be explained by the anti-oxidative properties of its constituents as well as the anti-apoptotic effects observed immunohistochemically in the current investigation which exhibited increased expression of GPX and subsequent decrease in the DNA fragmentation and caspase 3activity. This work was looked at the ability of pineapple extracts to protect rat ovaries against gamma radiation-induced cell death, oxidative stress, and hormonal alterations.

5. Conclusion

The effects of radiation on ovarian structure and function, as well as the expression of antioxidant enzymes, were discovered in female Albino rats in this study. Consumption of pineapple extract and bromelain, on the other hand, was found to be efficient in reducing the in toxicity and cellular damage caused by gamma radiation. Thus, the main findings imply that both the pineapple extract and bromelain are potent dietary antioxidants that can be used as exogenous supplementation to combat the oxidative stress and can improve reproductive health.

Declaration of Competing Interest

The authors report no declarations of interest.

Abbreviations

catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), lactate dehydrogenase (LDH), follicle-stimulating hormone (FSH), estrogen (E2), malondialdehyde (MDA), reactive oxygen species (ROS), radiotherapy (RT), granulosa cells (GCs), *p*-nitroaniline (pNA), caspase-3 (CPP32)

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