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Research Article

The effects of aerobic exercise and gallic acid on prostate cancer-related autophagy pathway genes in rats

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Abstract

Prostate cancer is one of the most common cancers. Regular physical activity and medicinal herbs can prevent their development by activating autophagy. However, the simultaneous effect of aerobic exercise and gallic acid on autophagic genes expression in prostate cancer tissues has not been studied. Accordingly, the present study determined the effect of aerobic exercise and gallic acid on the expression of Beclin-1, ATG5 and LC3 genes in the prostate tissue of male rats. In an experimental study, 60 male Wistar rats were randomly divided into 6 groups including 1-control-healthy group, 2-sham group, 3-control-prostate cancer group, 4-prostate cancer-aerobic exercise group, 5-prostate cancer-gallic acid group and 6-prostate cancer-aerobic exercise-gallic acid group. After prostate cancer was induced by inducing LNCaP and TSP-1-ENSCs cell lines, the subjects underwent aerobic exercise and gallic acid administration for eight weeks. At the end of the eighth week, the rats were sacrificed and their prostate tissue was removed. This was done to measure the expression of Beclin-1, ATG5 and LC3 genes by Real-Time PCR. Expression of BECLIN1 (P=0.019), ATG5 (P=0.001) and LC3 (P=0.001) genes was significantly lower in the control-prostate cancer induction group than in the control-healthy group. The expression of BECLIN1, ATG5 and LC3 genes was significantly higher in the aerobic exercise-prostate cancer group, the gallic acid-prostate cancer group and the aerobic exercise-gallic acid-prostate cancer group than in the control-prostate cancer group. According to the study results, it is concluded that aerobic exercise and gallic acid promote autophagy in prostate cancer tissue. It seems that the combination of these two interventions can be used as an effective strategy for managing and preventing disease progression.

Keywords:
Aerobic exercise, gallic acid, Beclin-1, ATG5, and LC3
Introduction

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1. Introduction

Prostate cancer (PCa) is common in Western populations and is the second leading cause of cancer death among men in North America (1). In recent years, the PCa incidence has also increased in Asian countries (2). According to the latest estimates, prostate cancer is becoming the most common cause of cancer-related death and poses a major public health concern, and new strategies for its prevention and treatment must be adopted. Evidence suggests that autophagy inhibits prostate cancer cell proliferation and survival. Furthermore, the metastasis of prostate cancer cells is influenced (through induction and inhibition) by autophagy (3). In cells, a wide range of physiological processes play unique roles in maintaining homeostasis and preventing pathological events Autophagy is a physiological cellular process for the degradation and elimination of misfolded proteins and damaged organelles that functions in adaptation to starvation, growth, cell death, and tumor suppression (4,5). One of the key mechanisms of autophagy is an intracellular degradation pathway mediated by double-membrane vesicles called autophagosomes. Autophagosomes deliver degraded cytoplasmic components to lysosomes for recycling under stressful conditions. This autophagy mechanism is essential for protecting cells from damaged proteins, protecting cellular organelles from toxins, maintaining cellular metabolism and energy homeostasis, and promoting cell survival (6). Autophagy has attracted much attention due to its dual role in cancer. Despite advances in prostate cancer treatment, it is critical to explore complementary and alternative medicine approaches to improve the health outcomes in prostate cancer patients.

Regular physical activity is considered a promising strategy for prostate cancer prevention and control (7). Available evidence suggests that regular physical activity is associated with reduced prostate cancer severity, inhibition of tumor progression and metastasis, and promotion of less aggressive cancer phenotypes (8,9). Physical activity levels are associated with reduced prostate cancer-specific mortality and disease progression (10). However, the molecular mechanisms underlying regular physical activity benefits against prostate cancer tumor growth and progression are still unclear. However, based on previous studies, evidence suggests that regular physical activity, especially aerobic exercise, can act as a modulator of autophagy and, consequently, has the potential to be used for cancer treatment (11). Regarding autophagy's role in cancer, it should be noted that its role is very complex (12). Autophagy suppresses tumors in the early stages. Therefore, increasing autophagy can prevent cancer (13). However, at advanced stages of cancer, autophagy is used by cancer cells to improve tumor structure and function. Therefore, inhibiting autophagy in cancer cells may improve cancer therapy (12). The anticancer effects of medicinal plants and their phytochemical compounds have long been recognized by researchers. Gallic acid, a natural plant phenolic compound, mediates various therapeutic properties that contribute to its anti-inflammatory, anti-obesity, and anti-cancer activities. Gallic acid has recently been shown to induce anticancer effects through several biological pathways, including migration, metastasis, apoptosis, cell cycle arrest, angiogenesis, and oncogene expression (14). Gallic acid is one of the most nutrient-rich natural polyphenols.

Swedish chemist Carl Wilhelm Scheele first isolated gallic acid from plants in 1786, when studies on its functions and its derivatives began (15). Gallic acid is a natural secondary plant metabolite with broad biological activities such as anti-inflammatory, antibacterial, antifungal, anti-ulcer, and anticancer (16). Gallic acid in cancer cells can induce apoptosis by affecting the expression of oncogenes, apoptotic and antiapoptotic proteins, matrix metalloproteinases (MMPs), ROS production, and targeting the cell cycle (17). Several studies have shown that gallic acid and its derivatives have anticancer effects on cancers such as prostate cancer, melanoma, leukemia, oral cancer, colon cancer, lymphoma, and breast cancer cells (18). In DU145 prostate cancer cells, gallic acid was the primary anticancer compound that suppressed cell growth. Gallic acid reduces the cell survival of DU145 cells by inducing mitochondrial and ROS-mediated apoptosis. Gallic acid also leads to cell cycle arrest in the G2/M phases by activating checkpoint kinase 1 and checkpoint kinase 2 (vital protein kinases that play an important role in the cellular response to DNA damage and cell cycle regulation) and inactivating Cell Division Cycle 25C and Cyclin-Dependent Kinase 1 (vital proteins in cell cycle regulation, especially during the transition from G2 to M phase (mitosis) (19). On the other hand, in PC3 prostate cancer cells, gallic acid can induce DNA damage and recruit several DNA repair genes (20). Gallic acid has also been reported to inhibit the migration and invasion of human PC3 prostate cancer cells (21). Since evidence shows that both aerobic exercise and gallic acid can reduce prostate cancer development by increasing autophagy. Based on the literature review, both interventions have not been studied simultaneously for their effect on autophagy-regulating genes.

Therefore, the present study aimed to determine the effect of aerobic exercise and gallic acid on the expression of Beclin-1, ATG5, and LC3 genes in the prostate tissue of rats with prostate cancer.

2. Materials and Methods

In an experimental study, 60 Wistar rats were obtained from the Laboratory Animal Breeding and Reproduction Center of Islamic Azad University, Marvdasht Branch. These animals were then kept in the Animal Exercise Physiology Laboratory of this university unit for one week to acclimate to the environment. Rats were kept under standard conditions of light, temperature 22 to 24°C, relative humidity 55 to 60%, 12-hour light and 12-hour dark cycle. They were kept in transparent, washable polycarbonate cages with free access to water and a special animal diet. The animals were randomly divided into 6 groups (10 rats per group) including 1-control group-healthy, 2-sham group, 3-control group-prostate cancer, 4-prostate cancer group-aerobic exercise, 5-prostate cancer group-gallic acid and 6-prostate cancer group-aerobic exercise-gallic acid. All ethical aspects of working with laboratory animals in this study were conducted in accordance with the Declaration of Helsinki.

Prostate cancer induction

To induce prostate cancer in rats, first LNCaP and TSP-1-EnSC cell lines were obtained from the Pasteur Institute of Iran under standard conditions and appropriate culture medium containing all nutrients for cell life. Subsequently, the cells were propagated and maintained in RPMI 1640 supplemented with 5% FBS manufactured by Life Technologies, Canada. To prepare a solution for injection into the prostate gland,

1 × 6 × 10 LNCAP cells were suspended in 75 µL of RPMI 1640, 5% FBS, and 75 µL of Matrigel (Collaborative Biomedical Products, Bedford, Massachusetts). Rats were anesthetized using ketamine and Xylazine. A transverse incision was made at the bottom of the abdominal cavity. After cutting the superficial and deep abdominal muscles, the bladder and seminal vesicles were released through the incision to expose the dorsal lobe of the prostate. The prepared suspension was carefully injected into the subcutaneous space on the right side of the prostate using a 27-gauge Hamilton needle. After this step, 4-0 absorbable sutures manufactured by Supa Company, Iran, were applied to close the wound. Also, OTC solutions were used to disinfect the sutures during the study period and after surgery (22).

Aerobic exercise protocol

First, rats with prostate cancer were familiarized with a treadmill specifically designed for rats and trained for one week. The familiarization was such that during this one week, the rats ran on a treadmill without an incline for 10 minutes a day. They ran at 8 meters per minute. According to McCullough et al. (2013), rats with prostate cancer were run for 60 minutes daily at 15 m/min on a 15-degree inclined. It is worth noting that training was performed for eight weeks and five sessions per week The activity time per session was 30 minutes in the first to fourth weeks and 60 minutes in the fourth to eighth weeks (23).

Gallic acid supplementation

Gallic acid was obtained from Sigma-Aldrich, USA. 20 mg/kg of gallic acid was administered daily to rats (24). The sacrifice and collection of tissues

Finally, 48 hours after the last training session and after a 12-hour fast, the rats were anesthetized using ketamine (55 mg/kg) and abdomen of the Rats were used. Lack of response to these tests was considered a sign of complete analgesia and anesthesia. Next, the rats' abdominal cavity was incised with a 20-gauge surgical blade. After removing other tissues and connective tissues, the rats' prostate tissue was carefully extracted. Prostate tissue was immediately placed in special tissue preservation microtubes and immersed in a nitrogen tank for 10 to 15 minutes. After immersion in the nitrogen tank, the tissues were transferred to -80°C until testing.

Measurement of variables by gene expression assay

Beclin-1, ATG5, and LC3 genes were expressed by the real time PCR method. For this purpose, a small amount of homogenized tissue was first used for RNA synthesis RNA synthesis was performed with a special kit manufactured by Qiagen, Germany. To assess RNA purity percentage, a spectrophotometric method with a wavelength of 260 nm was applied. If the concentration was desirable, the samples were used for cDNA synthesis by a special extraction kit manufactured by ROSH, Germany. The samples were combined with pre-designed primers, whose efficiency had been previously evaluated on the PUBMED site, and the samples were read. At the end of the different temperature and time cycles of the device, the variable values were evaluated along with the internal control gene based on the threshold cycle (Ct). The formula 2 - ΔΔCt was used to quantify the data.

Statistical Model

All the data are reported based on mean and standard deviation. First, to determine the effect of prostate cancer induction on the outcomes studied, healthy control groups, a sham healthy group, and prostate cancer control groups were analyzed using a one-way ANOVA. If a significant difference was observed, the groups were compared pairwise by the Tukey test to determine the location of the difference.

Two-way variance analysis was used to test the hypothesis. Based on this model, the effect of aerobic exercise and gallic acid alone on the outcomes under study was first evaluated. Aerobic exercise and gallic acid were tested. The study groups were compared using the Bonferroni post-hoc test. The significance level was also considered in all calculations ($p < 0.05$). All calculations were performed with SPSS version 25 software.

3. Results

The prostate cancer induction control group and healthy control group were compared, and it was found that the expression of BECLIN1 (P=0.019, d=1.93), ATG5 (P=0.001, d=2.83), and LC3 (P=0.001, d=2.56) genes was significantly lower in the prostate cancer induction control group compared to the healthy control group. No significant difference was observed in the expression of BECLIN1 (P=1.000, d=0.38), ATG5 (P=1.000, d=0.37) and LC3 (P=1.000, d=0.26) genes between the healthy-control and healthy-sham groups. Figure 1

In comparison with the placebo group (figure and table 2), an individual analysis of lactic acid confirmed a significant difference in the two groups of caffeine (p=0.039) and sodium bicarbonate+caffeine (p=0.031). An individual analysis of ammonia levels in blood samples confirmed that all three experimental groups of caffeine (p=0.044), sodium bicarbonate (p=0.046) and sodium bicarbonate+caffeine (p=0.038) had significantly different ammonia levels compared to the placebo group (table 2). There were no significant differences in ammonia, lactic acid or glucose levels between the three experimental groups (p≥0.05).

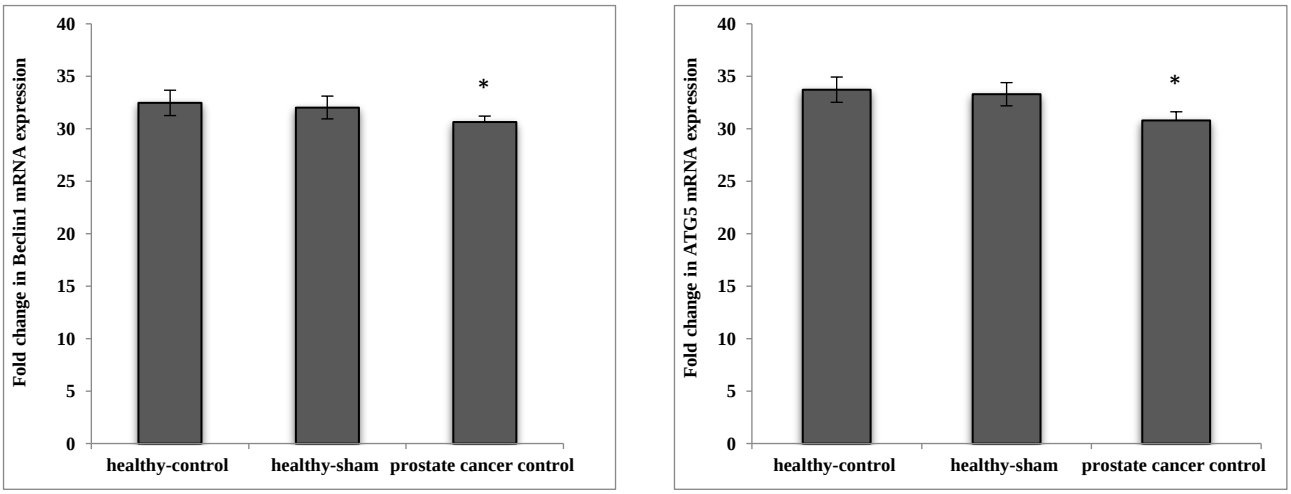
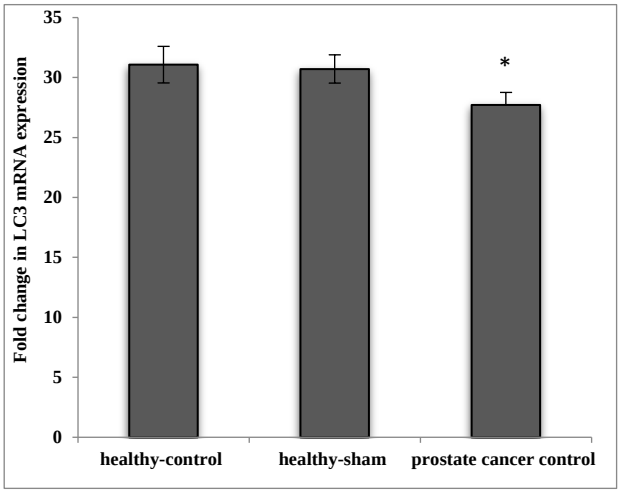


Figure 1- Comparison of BECLIN1, ATG5 and LC3 gene expression in prostate tissue in a healthy control group, healthy sham group and prostate cancer control group. *Significant differences compared to a healthy control group and a healthy sham group. Data are reported based on mean and standard deviation.

The expression of BECLIN1 gene in the aerobic exercise-prostate cancer group (P=0.001, d=2.90), gallic acid-prostate cancer group (P=0.001, d=3.18), and aerobic exercise-gallic acid-prostate cancer group (P=0.001, d=5.02) was significantly higher than in the control-prostate cancer group. Although the highest expression of BECLIN1 gene in prostate tissue was observed in the aerobic exercise and gallic acid group, the simultaneous effect or, in other words, the interaction of these two interventions on the expression of this gene was not statistically significant. Figure 2



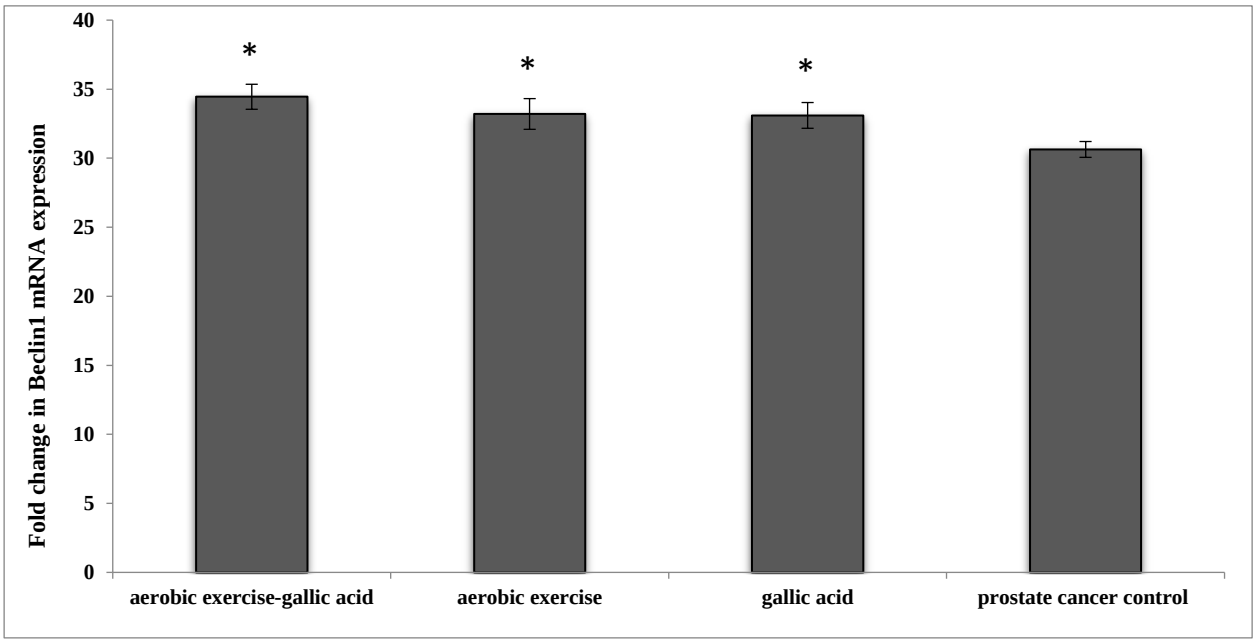


Figure 2- BECLIN1 gene expression in prostate tissue in the study groups. * Indicates a significant difference compared to the control group for prostate cancer. Data are reported based on the mean and standard deviation.

The expression of ATG5 gene in the aerobic exercise-prostate cancer group (P=0.005, d=1.59), the gallic acid-prostate cancer group (P=0.001, d=2.03), and the aerobic exercise-gallic acid-prostate cancer group (P=0.001, d=3.42) was significantly higher than the control-prostate cancer group. Although the highest expression of ATG5 gene in prostate tissue was observed in the aerobic exercise and gallic acid group, the simultaneous effect or, in other words, the interaction of these two interventions on the expression of this gene was not statistically significant. Figure 3

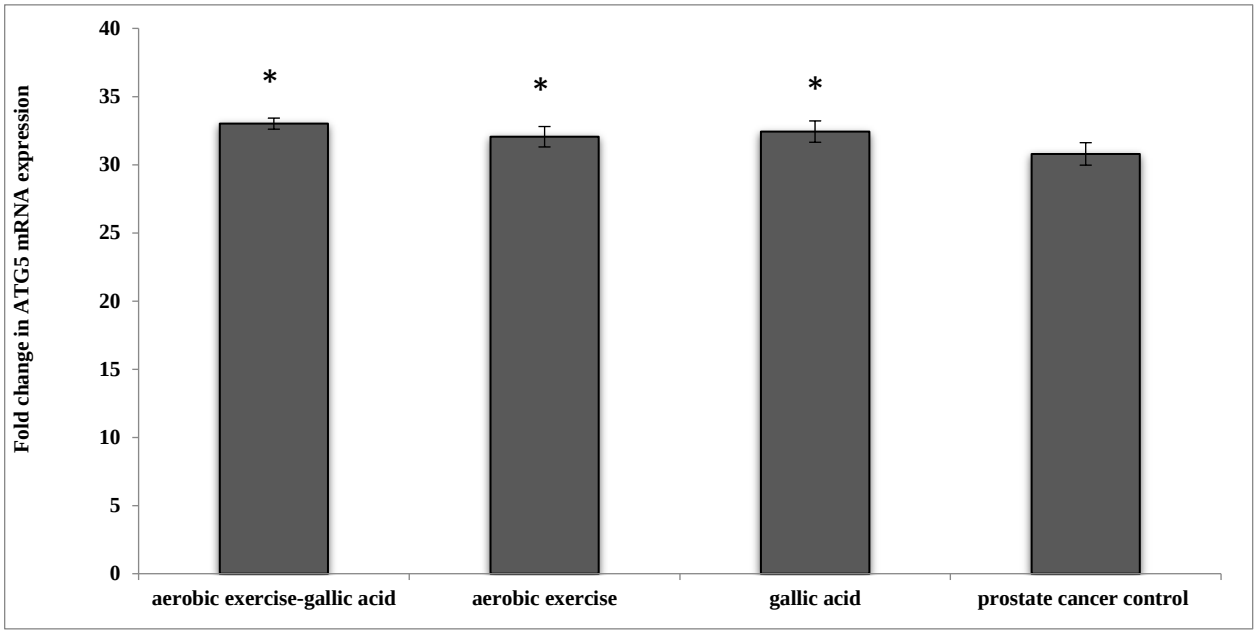


Figure 3 - ATG5 gene expression in prostate tissue in the study groups. * Indicates a significant difference compared to the control group with prostate cancer. Data are reported based on the mean and standard deviation.

LC3 gene expression in the aerobic exercise-prostate cancer group (P=0.002, d=2.73), the gallic acid-prostate cancer group (P=0.006, d=2.58), and the aerobic exercise-gallic acid-prostate cancer group (P=0.001, d=2.07) was significantly higher than in the control-prostate cancer group. Although the highest expression of LC3 gene in prostate tissue was observed in the aerobic exercise and gallic acid group, the simultaneous effect or, in other words, the interaction of these two interventions on the expression of this gene was not statistically significant. Figure 4.

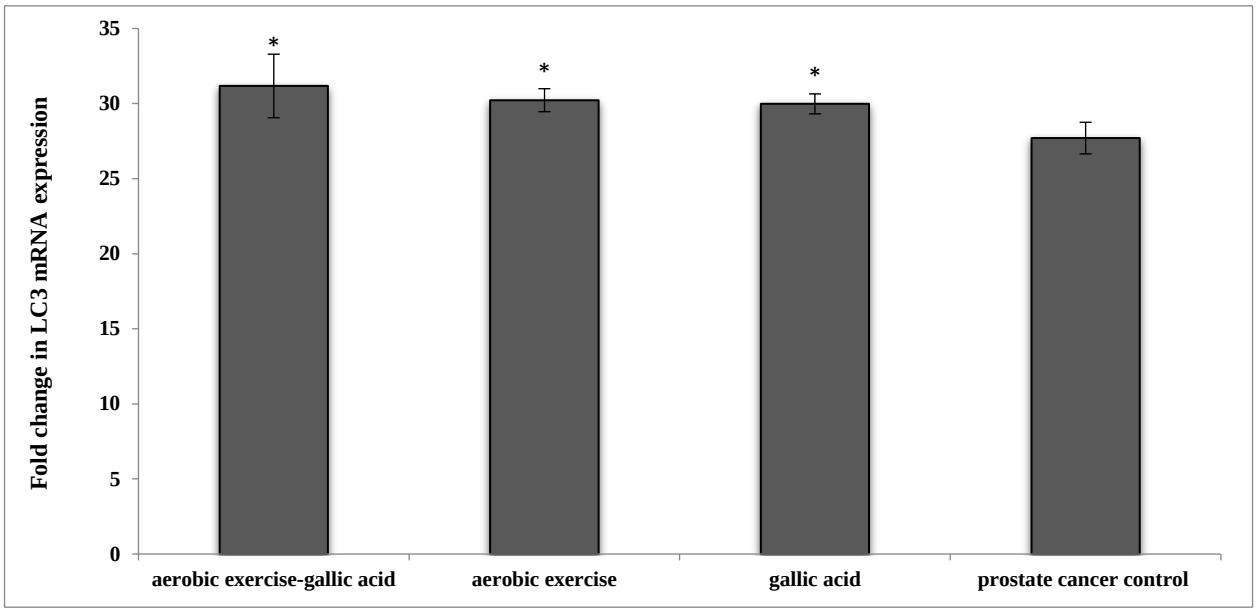


Figure 4- LC3 gene expression in prostate tissue in the study groups. * Indicates a significant difference compared to the control group with prostate cancer. Data are reported based on mean and standard deviation.

4. Discussion

The first finding of the present study showed that induction of prostate cancer resulted in a decrease in the expression of BECLIN1, ATG5, and LC3 genes. Evidence from cancer biology suggests that autophagy plays a dual role in tumor promotion and suppression and contributes to cancer cell growth and proliferation (25,26). Preclinical studies have shown a dual role for autophagy in prostate cancer survival and proliferation. Some anti-cancer drugs regulate autophagy. Therefore, autophagy-regulated chemotherapy could contribute to cancer cells' survival or death (27, 28). Like all cellular regulatory processes, autophagy is induced or inhibited by proteins. Autophagy regulation helps express tumor suppressor proteins or oncogenes. One of the key proteins in autophagy is Beclin1. Beclin1 is a key protein in autophagy induction by interacting with Vps34 to form the Beclin1-ATG14-Vps34-Vps15 complex, which is essential for the localization of autophagic proteins to the site of autophagosome formation. Beclin1, through its role in initiating autophagy, has been strongly associated with cancer and has both tumor-suppressive and tumor-promoting functions.

Previous studies have reported that downregulation of the autophagy-related gene Beclin1 (encoding Beclin1) is observed in a variety of human breast, prostate, and ovarian cancers (29, 30). Beclin1's role in phagophore formation is critical and suggests that Beclin1 functions as a tumor suppressor. In cancer cell lines and mouse models, loss of Beclin1 resulted in decreased autophagy and increased cell proliferation. This indicates that the Beclin1 gene functions as a tumor suppressor (31).In addition, several studies have shown that Beclin1 levels are reduced in various cancers, such as cervical squamous cell carcinoma and hepatocellular carcinoma (32, 33, 34). In the present study, Beclin1 gene expression was reduced in prostate cancer groups, which is consistent with previous studies that showed that allelic loss of the Beclin1 autophagy gene is highly observed in breast, ovarian, and prostate tumors, and this reduction is observed with an increase in the incidence of tumor formation in Beclin 1+/− mutant mice. These findings indicate the role of Beclin1 and autophagy in tumor suppression (1,35). The reduction in Beclin1 gene expression in prostate cancer appears to be primarily associated with epigenetic changes, particularly DNA methylation.

In prostate cancer cells, hypermethylation of the Beclin1 promoter region can lead to reduced gene transcription, disrupting its role in autophagy and tumor suppression (36). In addition, other regulatory mechanisms, such as changes in transcription factors and signaling pathways that affect Beclin1 expression, may contribute to its downregulation in prostate cancer. Consistent with the downregulation of Beclin1 gene expression, the expression of autophagy-related gene 5 (ATG5) was also significantly reduced after prostate cancer induction. In mammals, ATG5 plays a regulatory role during the transition from autophagy to apoptosis, and the molecular regulatory mechanisms involved in this protein have been elucidated (37). ATG5 may promote autophagosome expansion by combining with ATG12 and ATG16 to form ATG12-ATG5-ATG16L complexes (38). Alternatively, ATG5 may be cleaved to produce NtATG5, which binds to the apoptosis inhibitor protein BCL-XL located in the mitochondrial membrane. This binding results in the activation of the pro-apoptotic protein BAX to form a BAX-BAX homodimer, enhancing apoptosis (37, 39). To date, 41 ATGs have been identified, and among these 41 proteins, ATG5 is essential for autophagic vesicle formation. Knockdown of ATG5 can lead to reduction or complete inhibition of autophagy, indicating that ATG5 plays a central role in autophagy (40). Therefore, ATG5 is one of the most common target genes in autophagy gene editing assays. In addition, ATG5 has other functions, including mitochondrial quality control after oxidative damage, adipocyte differentiation, and apoptosis (41). ATGs regulate autophagosome elongation. ATG5-ATG12/ATG16L complexes recruit microtubule-associated protein 1 light chain 3 (LC3) and are associated with phagophore expansion (42). In mice deficient in autophagic core proteins, deletions of ATG5 and ATG7 have been reported to cause liver cancer due to damaged mitochondria and oxidative stress (43). Other studies have shown that a deficiency of autophagy regulators, such as ATG3, ATG5, ATG9, is associated with oncogenesis (44,45). Also, reduced expression of ATG5, a key regulator of autophagy, decreased survival in 158 primary melanoma patients

Reduced ATG5 expression enhanced cancer cell proliferation and was associated with early cancer progression (46). In support of the role of autophagic proteins, especially ATG5, in cancer suppression, it has been reported that knockdown of autophagy-related proteins, such as Beclin 1, ATG5, and ATG7, increases migration and invasion by regulating EMT in glioblastoma cells (6). Evidence suggests that various mechanisms contribute to ATG5 gene expression downregulation in prostate cancer. One of these mechanisms is hypermethylation of the ATG5 promoter region. Methylation can inhibit the binding of transcription factors required for gene expression and decrease ATG5 levels (47). Alterations in the expression or activity of transcription factors that regulate ATG5 expression can also contribute to its downregulation. For example, factors that normally increase ATG5 expression may be suppressed in prostate cancer cells. Some microRNAs (miRNAs) can degrade the ATG5 mRNA or prevent translation, leading to reduced protein levels. As a result, specific miRNAs may be altered in prostate cancer and contribute to ATG5 downregulation. Prostate cancer may involve dysregulation of various signaling pathways (such as the PI3K/Akt/mTOR pathway) that affect autophagy and, consequently, the expression of autophagy-related genes such as ATG5 (48). Overall, reduced ATG5 expression in prostate cancer may contribute to tumor progression by allowing cancer cells to escape autophagy-induced cell death, thereby increasing their survival and proliferation. Furthermore, dysregulation of autophagy-related genes, including ATG5, could affect prostate cancer cells' response to therapies, making it a crucial area of research for understanding the disease and developing potential treatments. The LC-3 gene expression was also significantly reduced in the present study due to cancer induction. This is consistent with previous studies (1). The LC3 gene, also known as MAP1LC3 (microtubule-associated protein 1 light chain 3), encodes a protein involved in autophagy. The protein microtubule-associated protein 1 light chain 3 (LC3) plays a vital role in the formation of autophagosomes, which encapsulate cellular debris and deliver it to lysosomes for degradation.

There are several LC3 isoforms, including LC3A, LC3B, and LC3C, each with distinct functions in autophagy and cellular homeostasis. The study of LC3 and its role in autophagy is significant in understanding various diseases, such as neurodegenerative disorders and cancer (49). The reduction in LC3 gene expression in prostate cancer can be attributed to various mechanisms, including epigenetic changes, transcriptional repression, and alterations in signaling pathways regulating autophagy. In prostatic cancer, the tumor microenvironment and oncogenic factors may lead to the downregulation of autophagy-related genes, such as LC3. Furthermore, mutations or alterations in the expression of transcription factors that normally increase LC3 expression can further contribute to its downregulation. Understanding these mechanisms is crucial for the development of targeted therapies that restore autophagic processes in prostate cancer cells. Another finding of the present study shows that the expression of BECLIN1, ATG, and LC3 genes, which are autophagy regulatory genes, was significantly higher in the aerobic exercise-prostate cancer group than in the control-prostate cancer group. Since increased hypermethylation of the BECLIN1 promoter region in cancer leads to a decrease in the expression of autophagy-regulated genes, it seems that in the present study, aerobic exercise reduced DNA methylation and, as a result, increased BECLIN1 gene expression. It is well established that one of the potential biological mechanisms underlying the epidemiological association between physical activity and reduced risk of various cancers is the reduction in DNA methylation (50). DNA methylation is primarily mediated by three known DNA methyltransferases (DNMT1, DNMT3a, and DNA3b) that catalyze the transfer of a methyl group from S-adenosyl methionine to DNA (51). From a theoretical point of view, it is possible that regular physical activity inhibits DNMT activity. This disrupts methylation processes and, consequently, prevents the attenuation of genes regulating cell signaling pathways that carry out vital cell activities. Evidence suggests that regular physical activity, especially aerobic exercise, may reduce DNA methylation in tumor suppressor genes and thereby prevent tumor progression with potential effects on cancer survival (52).

Aerobic exercise affects various biological processes, including DNA methylation regulation, a key epigenetic mechanism that influences gene expression. While the specific mechanisms by which aerobic exercise reduces DNA methylation of autophagy genes in cancer are still under investigation, several potential pathways have been identified. One such mechanism is an increase in demethylation enzyme expression. Aerobic exercise may increase DNA demethylation enzyme expression, such as ten-eleven transferases (TETs). These enzymes convert 5-methylcytosine to 5-hydroxymethylcytosine, leading to a decrease in DNA methylation levels (53). Aerobic exercise, on the other hand, alters cancer tissues' metabolic status. Exercise increases the levels of certain metabolites that affect DNA methylation. For example, aerobic exercise promotes the availability of α-ketoglutarate, which is a cofactor for TET enzymes and may promote demethylation (54, 55). Overall, aerobic exercise appears to enhance the expression of autophagic genes BECLIN1, ATG, and LC3 in prostate cancer tissues. This is done by decreasing DNA methylation and increasing AMPK activity. This may inhibit tumor development and growth. Overall, studies in the field of prostate cancer and aerobic exercise suggest that regular aerobic exercise can enhance autophagy, which may improve cellular function and reduce inflammation. . In prostate cancer, some studies suggest that increased autophagy may inhibit tumor growth and improve treatment response. While the relationship between aerobic exercise, autophagy, and prostate cancer is still investigated, regular physical activity is generally recommended for overall health. It may protect against various cancer types, including prostate cancer. Another finding of the present study showed that gallic acid induction increased the expression of autophagy-activated genes including BECLIN1, ATG5, and LC3. This finding indicates that gallic acid prevents prostate cancer by stimulating autophagy. Gallic acid has antioxidant properties, but it can also induce mild oxidative stress in cancer cells. Oxidative stress stimulates autophagy as a protective mechanism. It can also lead to the upregulation of genes ATG5, Beclin1, and LC3 to manage cellular stress and maintain homeostasis. On the other hand, gallic acid may affect autophagy transcription factors.

For example, it can increase the expression of transcription factors such as TFEB (transcription factor EB). This promotes ATG5 genes and facilitates autophagy (56). Gallic acid in cancer cells can induce apoptosis by affecting the expression of oncogenes, apoptotic and antiapoptotic proteins, matrix metalloproteinases (MMPs), ROS production, and cell cycle targeting (57). Several studies have shown that gallic acid and its derivatives have anticancer effects on cancers such as prostate cancer, melanoma, leukemia, oral cancer, colon cancer, lymphoma, and breast cancer cells (58). In DU145 prostate cancer cells, gallic acid was the primary anticancer compound that suppressed cell growth. Gallic acid reduced DU145 cell survival by inducing mitochondrial and ROS-mediated apoptosis. Gallic acid also leads to cell cycle arrest in the G2/M phase by activating checkpoint kinase 1 and checkpoint kinase 2 (vital protein kinases that play an important role in the cellular response to DNA damage and cell cycle regulation) and inactivating Cell Division Cycle 25C and Cyclin-Dependent Kinase 1 (vital proteins in cell cycle regulation, especially during the transition from G2 to M phase (mitosis)) (59). On the other hand, in PC3 prostate cancer cells, gallic acid can induce DNA damage and recruit several DNA repair genes (60). Gallic acid has also been reported to inhibit the migration and invasion of human PC3 prostate cancer cells (61).

5. Conclusion

The results of the present study showed that prostate cancer induction was associated with a significant decrease in the expression of autophagic genes including Beclin-1, ATG5, and LC3 in prostate cancer cells. It seems that cancer cells have decreased autophagy function, one of the reasons for cancer growth and development. However, aerobic exercise reduced the inhibitory and destructive effects of cancer induction on the autophagy pathway. This was done by increasing the expression of the Beclin-1, ATG5, and LC3 genes. Gallic acid induction, like aerobic exercise, also had a positive effect on the autophagy pathway genes**.**

Although the greatest effect was observed when aerobic exercise and gallic acid were combined, the two interventions added to each other's effects on gene expression. Accordingly, it is concluded that both aerobic exercise and gallic acid independently and non-progressively (the sum of both interventions) affects the expression of these genes. These findings indicate that exercise and gallic acid can regulate autophagy. However, it seems that these effects occur independently of each other and without direct interaction with other signaling pathways. Ultimately, this study can be used as a foundation for future research investigating the complex interactions between aerobic exercise, natural compounds such as gallic acid, and autophagy processes. This is in the context of prostate cancer. Given the positive effects of aerobic exercise and gallic acid on autophagy and their contribution to inhibiting prostate cancer, the combination of these two interventions can be used as an effective strategy for managing and preventing disease progression.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: F.J, H.M.H, M.A.A ; Methodology: F.J, H.M.H, M.A.A.; Software: F.J, H.M.H, M.A.A.; Validation: F.J, H.M.H, M.A.A.; Formal analysis: F.J, H.M.H, M.A.A; Investigation: F.J, H.M.H, M.A.A.; Resources: F.J, H.M.H, M.A.A; Data curation: F.J, H.M.H, M.A.A; Writing - original draft: F.J, H.M.H, M.A.A; Writing - review & editing: F.J, H.M.H, M.A.A.; Visualization: F.J, H.M.H, M.A.A; Supervision: F.J, H.M.H, M.A.A; Project administration: M F.J, H.M.H, M.A.A.; Funding acquisition: F.J, H.M.H, M.A.A.

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