EURASIAN JOURNAL of MOLECULAR & BIOCHEMICAL SCIENCES

(EURASIAN MOL BIOCHEM SCI)

e-ISSN: 2822-4019

Volume : 1 - Number: 1 - March/April 2022

https://dergi.erzurum.edu.tr/ejmbs

Eurasian Journal of Molecular and Biochemical Sciences

Official Journal of Erzurum Technical University Faculty of Science

All articles in this journal are available from https://dergi.erzurum.edu.tr/ejmbs

Eurasian Mol Biochem Sci is published twice a year (March/April and September/October).

Indexing

Eurasian Journal of Molecular and Biochemical Sciences (Eurasian Mol Biochem Sci) is indexed by *Crossref, Index Copernicus, Google Scholar*.

Eurasian Journal of Molecular and Biochemical Sciences

Official Journal of Erzurum Technical University Faculty of Science

Year	Volume	Issue / Number
2022	1	1

ORIGINAL ARTICLES

Effect of Bortezomib administration on autophagic cell death in colorectal cancer cells

Elif Erbaş, Nevra Aydemir Celep, Kader Çiftci

Eurasian Mol Biochem Sci, 2022; 1(1): 1-5 (DOI 10.54672/ejmbs.2022.1)

Genotypic difference in response of chlorophyll content and peroxidase activities to salt stress in triticale

Pınar Uysal, Sinan Ata

Eurasian Mol Biochem Sci, 2022; 1(1): 5-9 (DOI 10.54672/ejmbs.2022.2)

Investigation of the effects of different genotypes on regeneration capacity in Triticale

Ismail Bezirganoglu Serap Karaman, Beyza Reisoğlu, Fatma Böke, Büşra Yazıcılar

Eurasian Mol Biochem Sci, 2022; 1(1): 10-15 (DOI. 10.54672/ejmbs.2022.3)

The effects of Quercetin administration on heart tissue and serum parameters in the eats with experimental obesity

Cihan Gür, Seçkin Özkanlar, Semin Gedikli, Emin Şengül, Volkan Gelen, Adem Kara

Eurasian Mol Biochem Sci, 2022; 1(1): 16-21 (DOI. 10.54672/ejmbs.2022.4)

Antimicrobial and antibiofilm activity of Ferula spp. growing in Erzurum

Şimal Topçu, Elif Arslan, Şeymanur Çobanoğlu, Ayşenur Yazıcı, Ümit İncekara

Eurasian Mol Biochem Sci, 2022; 1(1): 22-27 (DOI. 10.54672/ejmbs.2022.5)

REVIEW

The potential role of long non-coding RNAs and micro RNAs in insects: From junk to luxury

Insha Shafi, Mudasir Gani, Sadiah Shafi, Mohd. Ayoub Mantoo, Kamlesh Bali, Rakesh Kumar Gupta

Eurasian Mol Biochem Sci, 2022; 1(1): 28-37 (DOI. 10.54672/ejmbs.2022.6)

Eurasian Journal of Molecular and Biochemical Sciences

Official Journal of Erzurum Technical University Faculty of Science

List of Referees and Advisors for This Issue ***(Vol:1, Issue:1, 2022)***
Doğan İlhan, Kafkas University
Erdal Elkoca, Ağrı İbrahim Çeçen University
Gözde Büşra Eroğlu, Erzurum Technical University
İrfan Çınar, Kastamaonu University
İsmail Bezirganoğlu, Erzurum Technical University
Mehmet Cemal Adıgüzel, Atatürk University
Murat Karameşe, Kafkas University
Ömer Faruk Karataş, Erzurum Technical University
Seda Yakut, Bingöl University
Selcen Çelik Uzuner, Karadeniz Technical University
Serkan Örtücü, Erzurum Technical University
Ümit İncekara, Atatürk University

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Effect of Bortezomib administration on autophagic cell death in colorectal cancer cells

Elif Erbaş[1*], Nevra Aydemir Celep[1], Kader Çiftci[2]

[1] *Atatürk University, Faculty of Veterinary Medicine, Department of Histology and Embriyology, 25240, Erzurum, Turkey*

[1] *Atatürk University, Faculty of Veterinary Medicine, 25240, Erzurum, Turkey*

Cite: Erbaş E, Aydemir Celep N, Çiftci K. Effect of Bortezomib administration on autophagic cell death in colorectal cancer cells. Eurasian Mol Biochem Sci 2022;1(1): 1-5.

Received: 28 December 2021, Accepted: 10 February 2022 *DOI 10.54672/ejmbs.2022.1*

Abstract

Colon cancer is the most common cancer type after breast and prostate cancer in humans. Bortezomib is a proteasome inhibitor and is commonly preferred for the treatment of some types of cancer due to its efficiency and lower side effects. This study has investigated the impact of Bortezomib on cell death regarding the stimulation of autophagy. Bortezomib (Velcade) was treated to colorectal cancer cells (HT-29) for 24 hours at different concentrations (10 nM, 20 nM, and 40 nM). MTT analysis was used to determine the viability of Bortezomib-treated HT-29 cells, and immunocytochemical methods were used to determine bortezomib's effects on the expression of Beclin-1 and LC3 levels in the HT-29 cells. In MTT analysis, viability was decreased with an increase in bortezomib concentration and the lowest viability was found at 40 nM concentration. In the study, Beclin-1 immune reactive cells were seen as higher in 10nM and 40 nM concentrations of Bortezomib than other groups. Additionally, in LC3 evaluation, the immune reactive cell density was the highest at 40 nM concentration of Bortezomib ($p<0.05$). However, the LC3 immune reactivity was higher at 20nM and 40 nM concentrations of bortezomib groups ($p<0.05$). The findings revealed that the treatment of Bortezomib leads to an increase in levels of LC3 and Beclin-1 and activate the autophagy in colon cancer cells.

Keywords: Cancer, Autophagy, Bortezomib, Beclin-1, LC3.

Introduction

Colon cancer is one of the most common cancers in men and women especially in developed western countries [1]. The mortality rate of colon cancer has decreased in recent years because of the better treatment possibilities. Current standard treatment of colon cancer includes tumor resection after chemotherapy and biologic therapy [2-7]. The standard chemo-therapy for colorectal cancer patients is 5-fluorouracil combined with either oxaliplatin or

* *Corresponding Author:* Elif Erbaş,
Atatürk University, Faculty of Veterinary Medicine,
Department of Histology and Embriyology,
25240, Erzurum, Turkey
E-mail: eliferbas@atauni.edu.tr

irinotecan [2,4,5,8]. Although large number of clinical studies focused on finding the most effective approach including these drugs, unfortunately advanced colorectal cancer remains unresectable and incurable. However, studies on new targets and new treatment approaches are continued [9].

Bortezomib is the first clinically available 26S proteasome inhibitor and is used in various cancer treatments [10-13]. Bortezomib mediates reversible binding of the catalytic core complex to the N-terminal threonine residue in the β-1 subunit. It also leads to reversible inhibition of the proteolytic activity of the proteasome. This triggers various biological changes such as cell cycle arrest as well as induction of autophagy and apoptosis [1].

Bortezomib is the first clinically available 26S proteasome inhibitor and is used in various cancer treatments [10-13]. Bortezomib mediates reversible binding of the catalytic core complex to the N-terminal threonine residue in the β-1 subunit. It also leads to reversible inhibition of the proteolytic activity of the proteasome. This triggers various biological changes such as cell cycle arrest as well as induction of autophagy and apoptosis [1].

Materials and Methods

Cell culture: Human colon cancer cell line HT-29 was obtained from Ankara Şap Enstitüsü. The cells were incubated with high-glucose DMEM (Gibco) supplemented with 10 % heat-inactivated fetal bovine serum, 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 mg/ml). HT-29 cells were cultured in 25 cm2 cell culture flasks (Corning) in a 5% CO2 incubator (Esco) at 37oC. Subcultures were detached with trypsin and then counted with the trypan blue counting method., The HT-29 cells were then seeded in 96 well plates with 100 µm DMEM for cytotoxicity analysis and seeded on the coverslips in the six-well plates with 2000 µm of DMEM. After 24h incubation, the culture media were removed; the cells were washed with PBS and MTT cell proliferation assays were performed.

Drug administration: Bortezomib application was performed with the 0, 5, 10, 20 and 40 nanomolar (nM) concentrations for MTT analysis and 0, 10, 20 and 40nM concentrations for immunocytochemical analysis. Human Colorectal cancer cells (HT-29) were seeded in a six-well plate at a density of 20000 cells/well on a coverslip with 2 ml of complete culture medium and overnighted to attach. After 24 h, bortezomib was diluted with DMEM solution and added to cell's media at the concentration of 0nM, 10nM, 20nM, and 40nM (Velcade by Millennium Pharmaceuticals Inc. USA). After 24 h incubation, the coverslips were taken, and the cells were fixed with methanol solution at -20 oC for 10 min and then washed with PBS in a six-well plate. After fixation immunocytochemical staining was performed in coverslips mounted to the slides.

Cell viability: Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma Co. USA, Cat No: M5655). For MTT analysis, the cells were seeded in a 96-well plate at a density of 100,000 per well in the 100 µm growth media for 24h. The cells were incubated with different concentrations of bortezomib (5nM, 10nM, 20nM, and 40nM) for 24h, then the cells were washed with PBS and then incubated with 1 ml of MTT solution (0.25 mg/ml in PBS) at 37oC for 4h. After incubation, the medium was removed and 1 ml of 0.1 mol/l HCl in absolute isopropanol was added. The absorbance was measured by a spectrophotometer at the wavelength of 570 nm. The absorbance measurements were done two times.

Stereological estimation of anti-Beclin-1 and LC-3 immune reactive cell densities: The "Stereological Optic Fractionator Frame" method was used to estimation of anti-Beclin-1 and LC-3 immune reactive cell densities. These analyzes were performed

under a stereology workstation system (BioPrecision MAC 5000controller system) and stereology software (Stereo Investigator version 9.0, Microbrightfield, Colchester, VT) attached to the light microscope (Leica DM4000 B, Tokyo, Japan).

In our study, anti-Beclin-1 and anti-LC-3 immune reactive cell densities in HT-29 cell preparations were calculated using the "Unbiased Counting Frame and Fractionator" method, and the positive cell density in each preparation belonging to all groups was calculated according to the following formula:

PHY = PHS/(CA x RS),

PHY; positive cell density per µm² area, PHS; positive cell count, CA; frame area (µm²) and RS; the number of frames. The data obtained are based on duplicate measurements for each group, and 4 parallel preparations from each group were stained. The results are expressed as immune reactive cells / 1000 µm².

Statistical analysis: Normally distributed values from immunocytochemical analyses were evaluated with Duncan Post hoc test after one-way analysis of variance (ANOVA) using SPSS statistical software version 20.0 (SPSS Inc., Chicago, IL, USA). The significant value (p) was accepted as 0.05.

Results

Cell Viability: MTT analysis revealed that cell viability was decreased with the increase of bortezomib concentration treated to HT-29 cells. The lowest toxicity was found at 5nM concentration treated HT-29 cells, the highest toxicity was found in 40nM concentration treated HT-29 cells (Figure 1).

The effect of bortezomib on the anti-Beclin-1 and LC-3 immune reactive cells in HT-29 cell line: In the stereological estimation of immune reactive cell analysis, there were significantly increased levels of Beclin-1 proteins after bortezomib treatment as 10nM, 20nM, and 40nM in HT-29 cells compared with untreated HT-29 cells ($p<0.05$). In addition, 40nM concentration of bortezomib was markedly increased in Beclin-1 immune reactive cells at 24 h incubation in HT-29 cells ($p<0.05$) (Table 1 and Figure 2).

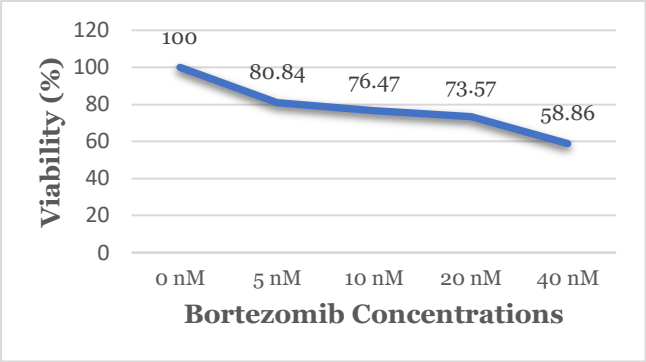


Figure 1. Cell viability values of HT-29 colon cancer cells treated with bortezomib for 24h using MTT analysis.

On the other hand, in LC-3 immune cells, the low dose of bortezomib (10nM) did not induce a significant difference ($p>0.05$). However, 20nM and 40nM concentrations of bortezomib increased the immune reactive cell density compared to 0nM and 10nM concentrations in the HT-29 cells. Beclin-1 and LC3 immune reactive cell densities are presented in Table 1 and demonstrated in Figure 2.

Table 1. The Stereological estimation of anti-Beclin-1 and anti-LC3 immune reactive cells in HT-29 colon cancer cells incubated with 0nM, 10nM, 20nM and 40nM concentration for 24h.

Groups	Anti-Beclin-1 positive cell density (n/1000 µm²)	Anti-LC3 positive cell density (n/1000 µm²)
Control	0.192±0.0032[a]	0.211±0.0038[a]
10-nM	0.342±0.0023[b]	0.292±0.0061[a]
20-nM	0.363±0.0062[b]	0.603±0.0042[b]
40-nM	0.447±0.0083[c]	0.641±0.0019[b]

Discussion

Bortezomib is a proteasome inhibitor and is widely used in many tumoral malignancies. Despite abundant evidence of the therapeutic potential of this drug, the relevant signaling pathways leading to autophagy in cancer cells were not clear. In this study, we demonstrated that bortezomib could induce autophagy via Beclin-1 and LC3 proteins in the HT-29 cell line. These results suggest that bortezomib could be a

potentially significant chemotherapeutic agent for the treatment of colorectal cancers.

Bortezomib is a specific inhibitor of the 26S proteasome [22] and it is an approved product for the treatment of multiple myeloma and acts as a prominent apoptosis inducer [23]. Most researchers report that bortezomib has anti-tumor properties that can be used in the treatment of many types of cancer [24]. In the treatment of colon cancer, surgical removal of solid tumor masses is usually required with appropriate chemical therapy. Chemotherapeutics such as bortezomib are triggered with the autophagy cell death via cell death receptor induction or disturbance of mitochondrial balance. Recently, the cell death mechanisms such as autophagy and necrosis are desired targets for therapeutic applications [1,25,26].

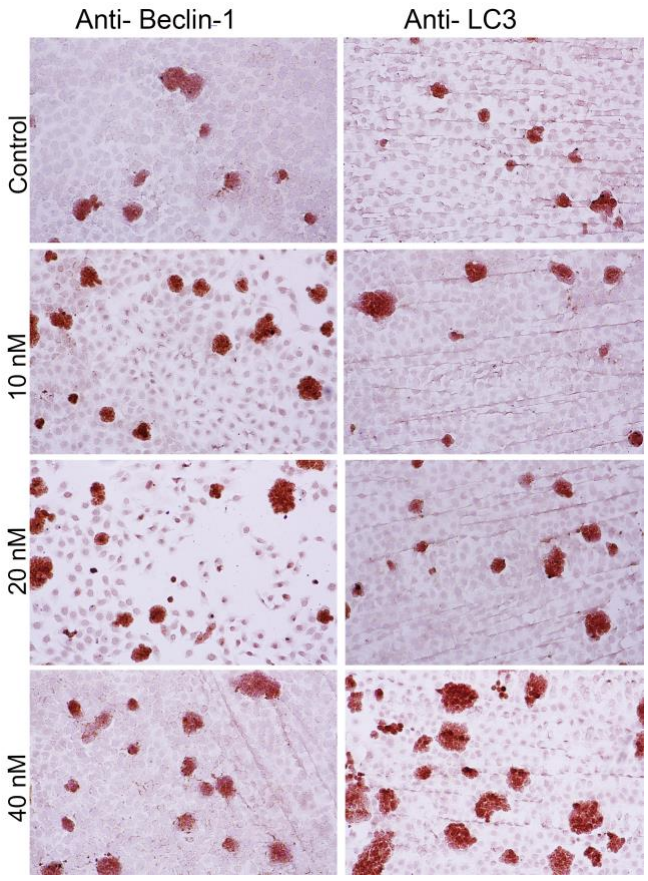


Figure 2. The Immunocytochemical illustration of anti-Beclin-1 and anti-LC-3 staining of HT-29 cells incubated with 0nM, 10nM, 20nM, and 40nM concentration of Bortezomib for 24h.

Despite its increasing therapeutic use for the treatment of many cancers, little is known about cytotoxicity-related mechanism of colon cancers [27]. Lou et al., suggested that the bortezomib may inhibit HOS cell proliferation through inhibition of ERK phosphorylation [14]. Hong et al., reported that bortezomib trigger G2/M arrest through intracellular reactive oxygen species-inducible ataxia telangiectasia mutated phosphorylation in colon cancer cells [28]. In our study, it was determined that increasing concentrations of bortezomib treatments decreased cell viability in the HT-29 cell line.

In this study, we examined the effectiveness of bortezomib to determine its potential effects on tumoricidal activity in colon cancer. Our data shown in Figure 2 presented that Beclin-1 and LC3 expression levels increased in a higher concentration of bortezomib treated groups (especially 10nM, 20nM, and 40nM) in a dose-dependent manner, once again confirming autophagy induction. Song et al., found that Beclin-1 and LC3-I expressions increased after 12 h from treatment [12]. On the other hand, bortezomib has also the potential to increase the Beclin-1, LC3-I, and LC3-II expression levels in different carcinoma cells such as hepatocellular carcinoma and multiple myeloma [17,29].

Several conclusions can be taken out from the data presented here. First, it was understood that the treatment of bortezomib induces autophagy and secondly, LC-3 and Beclin-1 play an important role in the autophagy activating the mitochondria-dependent pathway. Third, Beclin-1 and LC3 levels increased in bortezomib treated colon cancer cells. Therefore, it can be said that bortezomib treatment induced the Beclin-1 mediated autophagy. Finally, the bortezomib treatment significantly inhibits colon cancer tumor growth.

In the light of the findings obtained in the study, it was concluded that bortezomib administration may induce autophagy activation by causing an increase in

LC3 and Beclin-1 levels in colon cancer cells, and thus cause cell death.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: EE and NAC contributed to the study conception and design. KÇ contribution to laboratory work. Writing the article (EE and NAC). All authors read and approved the final manuscript. EE: Elif Erbaş, NAC: Nevra Aydemir Celep, KÇ: Kader Çiftci

ORCID

Elif Erbaş 0000-0003-1750-3889
Nevra Celep Aydemir 0000-0003-1608-5881

References

1. Kim SY, Song X, Zhang L, Bartlett DL, Lee YJ. Role of Bcl-xL/Beclin-1 in interplay between apoptosis and autophagy in oxaliplatin and bortezomib-induced cell death. Biochem Pharmacol 2014;88:178-88.
2. Hong YS, Hong SW, Kim SM, Jin DH, Shin JS, Yoon DH, et al. Bortezomib induces G2-M arrest in human colon cancer cells through ROS-inducible phosphorylation of ATM-CHK1. Int J Oncol 2012;41:76-82.
3. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337-45.
4. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355:1041-7.
5. Hochster HS, Hart LL, Ramanathan RK, Childs BH, Hainsworth JD, Cohn AL, et al. Safety and efficacy of oxaliplatin and fluoropyrimidine regimens with or without bevacizumab as first-line treatment of metastatic colorectal cancer: results of the TREE Study. J Clin Oncol 2008;26:3523-9.
6. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009;27:663-71.
7. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408-17.
8. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000;18:2938-47.
9. Pitts TM, Morrow M, Kaufman SA, Tentler JJ, Eckhardt SG. Vorinostat and bortezomib exert synergistic antiproliferative and proapoptotic effects in colon cancer cell models. Mol Cancer Ther 2009;8:342-9.
10. Fennell DA, Chacko A, Mutti L. BCL-2 family regulation by the 20S proteasome inhibitor bortezomib. Oncogene 2008;27:1189-97.
11. Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G. Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. Cell Cycle 2006;5:2592-601.
12. Song X, Kim SY, Zhang L, Tang D, Bartlett DL, Kwon YT, et al. Role of AMP-activated protein kinase in cross-talk between apoptosis and autophagy in human colon cancer. Cell Death Dis 2014;5:e1504.
13. Zhu K, Dunner K, Jr., McConkey DJ. Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene 2010;29:451-62.
14. Lou Z, Ren T, Peng X, Sun Y, Jiao G, Lu Q, et al. Bortezomib induces apoptosis and autophagy in osteosarcoma cells through mitogen-activated protein kinase pathway in vitro. The Journal of international medical research, 2013; 41(5): 1505-1519.
15. Di Lernia G, Leone P, Solimando AG, Buonavoglia A, Saltarella I, Ria R, et al. Bortezomib Treatment Modulates Autophagy in Multiple Myeloma. Journal of clinical medicine,2020; 9(2): 552.
16. Kim SY, Song X, Zhang L, Bartlett DL, Lee YJ. Role of Bcl-xL/Beclin-1 in interplay between apoptosis and autophagy in oxaliplatin and bortezomib-induced cell death. Biochemical pharmacology, 2014; 88(2): 178–188.
17. Hui B, Shi YH, Ding ZB, Zhou J, Gu CY, Peng YF, et al. Proteasome inhibitor interacts synergistically with autophagy inhibitor to suppress proliferation and induce apoptosis in hepatocellular carcinoma. Cancer 2012;118:5560-71.
18. Kao C, Chao A, Tsai CL, Chuang WC, Huang WP, Chen GC, et al. Bortezomib enhances cancer cell death by blocking the autophagic flux through stimulating ERK phosphorylation. Cell Death Dis 2014;5:e1510.
19. Mizushima N, Levine B. Autophagy in mammalian development and differentiation. Nat Cell Biol 2010;12:823-30.
20. Ding ZB, Hui B, Shi YH, Zhou J, Peng YF, Gu CY, et al. Autophagy activation in hepatocellular carcinoma contributes to the tolerance of oxaliplatin via reactive oxygen species modulation. Clin Cancer Res 2011;17:6229-38.
21. Shi YH, Ding ZB, Zhou J, Hui B, Shi GM, Ke AW, et al. Targeting autophagy enhances sorafenib lethality for hepatocellular carcinoma via ER stress-related apoptosis. Autophagy 2011;7:1159-72.
22. Karin M, Yamamoto Y, Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004;3:17-26.
23. Hideshima T, Chauhan D, Richardson P, Mitsiades C, Mitsiades N, Hayashi T, et al. NF-kappa B as a therapeutic target in multiple myeloma. J Biol Chem 2002;277:16639-47.
24. Green DR, Kroemer G. Pharmacological manipulation of cell death: clinical applications in sight? J Clin Invest 2005;115:2610-7.
25. Amaravadi RK, Lippincott-Schwartz J, Yin XM, Weiss WA, Takebe N, Timmer W, et al. Principles and current strategies for targeting autophagy for cancer treatment. Clin Cancer Res 2011;17:654-66.
26. Waters JP, Pober JS, Bradley JR. Tumour necrosis factor and cancer. J Pathol 2013;230:241-8.
27. Coquelle A, Mouhamad S, Pequignot MO, Braun T, Carvalho G, Vivet S, et al. Cell cycle-dependent cytotoxic and cytostatic effects of bortezomib on colon carcinoma cells. Cell Death Differ 2006; 13(5): 873–875.
28. Hong YS, Hong SW Kim SM. Bortezomib induces G2-M arrest in human colon cancer cells through ROS-inducible phosphorylation of ATM-CHK1. Int J Oncol 2012; 41: 76–82.
29. Hoang B, Benavides A, Shi Y, Frost P, Lichtenstein A. Effect of autophagy on multiple myeloma cell viability. Mol Cancer Ther 2009;8:1974-84.

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Genotypic difference in response of chlorophyll content and peroxidase activities to salt stress in triticale

Pınar Uysal[1*], Sinan Ata[2]

[*1]*Ministry of Food, Agriculture and Livestock, Eastern Anatolia Agricultural Research Institute 25090 Erzurum, Turkey*

[2]*Ataturk University, Faculty of Agriculture, Department of Soil and Plant Nutrition Erzurum, Turkey*

Cite: Uysal P, Ata S. Genotypic difference in response of chlorophyll content and peroxidase activities to salt stress in triticale. Eurasian Mol Biochem Sci 2022;1(1): 6-9.

Received: 17 January 2022, Accepted: 5 March 2022 *DOI* 10.54672/ejmbs.2022.2

Abstract

The goal of the present study was to determine the salinity resistance of 5 triticale genotypes. Salt-stress experiments were carried out under tissue culture conditions. Regenerated seedlings of five genotypes were exposed to 0 and 100 mM NaCl. Peroxidase activity and pigment content were determined for seedling applied with salt for 7, 14, and 21 days. Peroxidase activity was observed in the triticale seedlings of salt stress in in-vitro conditions. Melez 2001 and Mikham 2002 were determined to be more responsive to stress severity than the other genotypes in terms of chlorophyll content. Our results showed that there was a positive correlation between chlorophyll content and 100 mM concentration salt stress in salt-resistant triticale genotypes.

Keywords: Genotypic difference, Chlorophyll content, Peroxidase activities, Triticale.

Introduction

Salinity is one of the most significant abiotic factors decreasing the development and crop yield of agronomically important plants (1). To better improve cultivars against salt stress rely on the concerted efforts by different factors such as tissue physiology, gene transformation, and breeding. The use of new cell biology tools for elucidating the regulation functions of salt-stress resistance is based on the control of specific stress-related mechanisms (2). Hence, tissue culture is a very useful technique for improving salt stress-resistant plants. When a plant is exposed to salt severity, numerous transcription factors are activated, resulting in promoted degrees of several biomolecules and secondary metabolites (3). Salinity can causes serious injury and subsequently results in oxidative damage of enzymatic activities. NaCl stress resistance requires activation of physiological and metabolic structures to protect the intact cell from damaging mechanisms (4). Many types of research have been

* *Corresponding Author:* Pınar UYSAL,
Ministry of Food, Agriculture and Livestock,
Eastern Anatolia Agricultural Research Institute,
25090 Erzurum, Turkey
E-mail: p5uysal@gmail.com

conducted on changes in enzyme activity in seedlings during stress conditions such as salt severity. Nevertheless, most of these studies have focused on salt-susceptible crops such as rice (5), chickpeas (6), or maize (7). A few reports are present on alters in peroxidase activity and pigment prediction during salt resistance in vitro, and its role in the improvement of salt resistance is not fully known. The goal of the present work was to determine the peroxidase activity and total pigment content of five triticale genotypes in terms of salt-stress resistance.

Material and Method

Salt-stress treatment: Seeds of 5 genotypes of triticale were provided by the East Anatolia Agricultural Research Institute, Erzurum. Mature seeds were sown in dishes (15 cm) containing the autoclaved half-strength MS medium and 0.6% (w/v) Phytagel (Sigma-Aldrich). Petri dishes (3 per treatment) contained 25 seeds each in growth chambers fluorescent light with 62 µmol m-2 s-1 and 16 h : 8 h light:dark cycle at 26 ± 1 °C. To determine seed germination in response to salt, seeds were sown in media containing 0 (control), and 100 mM NaCl. Seedlings were evaluated for accumulation of total pigment estimation and peroxidase enzyme activity.

Enzyme Activity: The POX activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5) containing 1mM guaiacol and 0.5 mM H2O2 (8). One unit of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01/min.

Pigment Estimation: Leaf chlorophylls (Chl) were extracted in 80% acetone and absorbance at 663 and 645 nm were measured. Chl a, Chl b, and total Chl contents were then calculated.

Statistical Analysis: Each experiment was repeated three times. Analysis of variance was conducted using the one-way ANOVA test using SPSS 13.0 and means were compared by the Duncan test at the 0.05 level of confidence.

Results

Peroxidase assay: Peroxidase increased in callus of all triticale cultivar under salt stress. A continuously increase in peroxidase was determined in stress time in all genotypes. The highest peroxidase value in 21 days was found in 'Tatlıcak' followed by 'Alper Bey', 'Mikham 2002', 'Melez 2001' and Ümran Hanım. Alper Bey and Ümran Hanım genotypes displayed a more increase in peroxidase dosage in 14. day, compared to 21. day (Figure 1). The highest peroxidase amount in 14 days, was also found in 'Tatlıcak', followed by 'Alper Bey', 'Ümran Hanım', 'Mikham 2001', and Melez 2001. Peroxidase values displayed a middle range of variation between genotypes in 7 days, ranging from 4.5 to 6.25 nmol g-1 FW (Figure 1). The lowest value of peroxidase in 7 days, was found in 'Ümran Hanım', and the lowest in both 'Mikham 2002' and 'Melez 2001'.

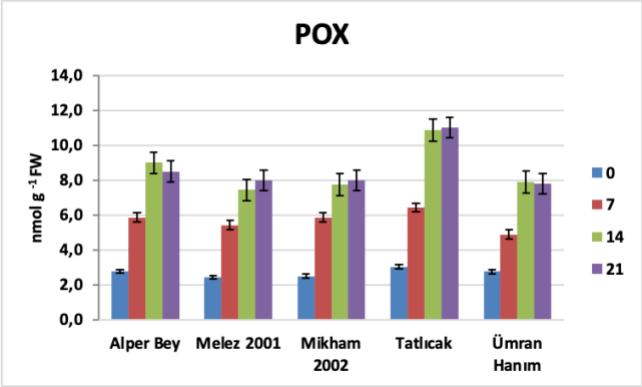


Figure 1. Changes of POX in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

Pigment Estimation: Chlorophyll content was different among triticale cultivars at salt stress. At salt stress, the highest chlorophyll content was obtained in Ümran Hanım, whereas the lowest was obtained in Mikham 2002 and Melez 2001 after 21 days at cold acclimation. Similarly, the same cultivars had the highest chlorophyll content at the salt stress for 7 and

14 days (Figure 2, 3, and 4).

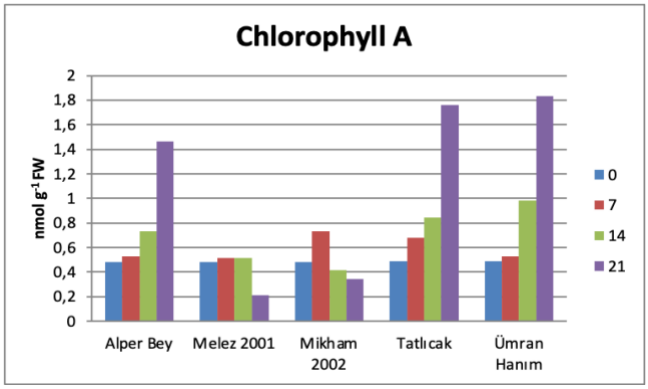


Figure 2. Changes of chlorophyll A in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

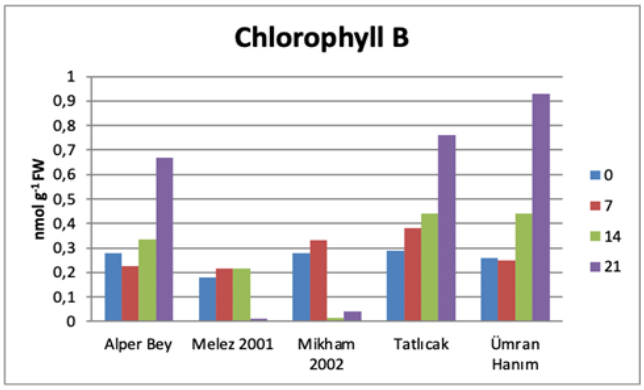


Figure 3. Changes of chlorophyll B in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

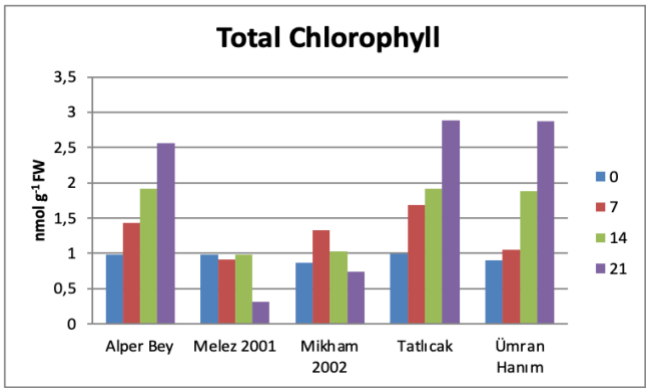


Figure 4. Changes of total chlorophyll in five triticale genotypes treated with 100 mM salt stress in in vitro conditions.

Discussion

In terms of salt stress mechanism, most of the studies reported so far have been conducted at the whole plant stage (6) however, this is the first showing the determination of salinity in triticale. Peroxidase enzymes play a significant role in the defense responses of crops to biotic or abiotic stresses (9). In this experiment, a considerable increase in the peroxidase activity could be determined in the seedlings of salt stress in in vitro conditions. Pujari and Chanda et al., (2002) (10) indicated that a salt-stressed seedling of Vigna unguiculata retained more peroxidase than that of unstressed seedling when both were applied with higher salt stress. Moreover, the activity of peroxidase in several species has been well documented in response to abiotic stress. The effects of stress severity on the peroxidase enzyme are proved to be linked to the salt-resistant ability (11). Our results displayed the highest degree of peroxidase in the high concentration salt-treated group. Thus it seems that this property is an effective indicator of salt-resistant in seedlings derived from Triticale cultivars. Chlorophyll is the basic factor for green pigments and is present in chloroplasts as components in all photosynthetic plant tissue. In this report, pigment estimation in seedlings was also influenced by NaCl (Figure 2, 3, and 4), and this effect depends on the genotypes. Compare to control groups, chlorophyll a, b and carotenoid content in two triticale (Melez 2001 and Mikham 2002) genotypes decreased (Figures 2, 3, and 4), whereas the other three triticale (Ümran Hanım, Alper Bey and Tatlıcak) genotypes increased after 1 week. The effect of inhibition of chlorophyll under stress conditions plays a critical role in photoinhibition or ROS formation (12). The decrease in photosynthesis under stress can also be attributed to a reduction in chlorophyll values. However, salinity decreases the chlorophyll content in salt-sensitive genotypes and promotes it in salt-resistant genotypes. In conclusion, these results displayed that there is a positive correlation between chlorophyll content and low concentration salt stress in salt-resistant triticale genotypes.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: PU and SA contributed to the study conception and design. PU and SA contribution to laboratory work. Literature research (PU and SA), Writing the article (PU and SA). All authors read and approved the final manuscript. PU: Pınar Uysal, SA: Sinan Ata

ORCID

Pınar Uysal 0000-0002-0998-4785
Sinan Ata 0000-0002-7905-4014

References

1. Misra, N., & Dwivedi, U. N. (2004). Genotypic difference in salinity tolerance of green gram cultivars. Plant Science, 166(5), 1135-1142.
2. Elmaghrabi, A. M., Ochatt, S., Rogers, H. J., & Francis, D. (2013). Enhanced tolerance to salinity following cellular acclimation to increasing NaCl levels in Medicago truncatula. Plant Cell, Tissue and Organ Culture (PCTOC), 114(1), 61-70.
3. Bray, E.A., Bailey-Serres, J., & Weretilnyk, E. 2000. Responses to abiotic stress. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. American Society of Plant Biologists, Waldorf, pp 1158–1203
4. Xiong, L., & Zhu. J.K. 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ. 25:131–139
5. Zeng, L. 2005. Exploration of relationships between physiological parameters and growth performance of rice (Oryza sativa L.) seedlings under salinity stress using multivariate analysis. Plant Soil, 268: 51-59.
6. Maliro, M.F.A., D. McNeil, B. Redden, J.F. Kollmorgen & C. Pittock. 2008. Sampling strategies and screening of chickpea (Cicer arietinum L.) germplasm for salt tolerance. Genet. Resour. Crop Evol., 55: 53-63.
7. Chinnusamy, V., Jagendorf, A. & Zhu. J.K. 2005. Understanding and improving salt tolerance in plants. Crop Sci., 45: 437-448.
8. Janda, T., Szalai, G., Rios-Gonzales, K., Veisa, O., & , E., 2003. Comparative study of frost tolerance and antioxidant activity in cereals. Plant Sci. 164, 301–306.
9. Luhova, L., Lebeda, A., Hedererová, D., & Pec, P. (2003). Activities of amine oxidase, peroxidase and catalase in seedlings of Pisum sativum L. under different light conditions. Plant soil and environment, 49(4), 151-157.
10. Pujari, D.S. & Chanda, S.V. 2002. Effect of salinity stress on growth, peroxidase and IAA oxidase activities in vigna seedlings. Acta Physiol Plant. 24:435-439
11. Bakardijieva, N.T., Christova, N.V., & Christov. K. 1996. Reaction of peroxidase from different plant species to increased temperature and the effect of cadmium and zinc ions. In: Plant peroxidases: Biochemistry and Physiology, Obinger C, Burner U, Ebermann R, Peneck, Greppin H (Eds), Univ of Geneva pp 345–351.
12. Kato, M., & Shimizu, S. (1985). Chlorophyll metabolism in higher plants VI. Involvement of peroxidase in chlorophyll degradation. Plant and cell physiology, 26(7), 1291-1301.

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Investigation of the effects of different genotypes on regeneration capacity in Triticale

Ismail Bezirganoglu[1*], Serap Karaman[1], Beyza Reisoğlu[1], Fatma Böke[1], Büşra Yazıcılar[1]

[1]*Department of Molecular Biology and Genetics, Erzurum Technical University, 25050 Erzurum, Turkey.*

Cite: *Bezirganoglu İ, Karaman S, Reisoglu B, Böke F, Yazıcılar B. Investigation of the effects of different genotypes on regeneration capacity in Triticale. Eurasian Mol Biochem Sci 2022;1(1): 10-15.*

Received: 09 March 2022, Accepted: 31 March 2022 *DOI. 10.54672/ejmbs.2022.3*

Abstract

Triticale (x Triticosecale Wittmack) is a synthetic amphiploid cereal that grows on about 3 million hectares in the world. It is grown mostly for forage or animal feed, although some triticale-based foods can be purchased at health food stores or are found in some breakfast cereals. Mature embryos of two triticale cultivars (Ümran Hanım and Melez 2001) were used as the resources explants. The effects of one auxin type (2,4-D) and three various concentrations, (4.0 mg/l; 8mg/l; 12.0mg/l) callus formation and plant regeneration were determined. Callus formation values were detected over 85% in three concentrations. In terms of results, the highest embryogenic callus formation rate was determined 46.91 % at 12 mg/l of Ümran Hanım dicamba. However, the lowest embryogenic callus formation rate was found with a value of 12.86% in Ümran Hanım's 2,4-D 8.0 mg/l hormone application. The highest regeneration capacity was determined at 12.09% at a dose of 12.0 mg/l of Mikham 2001 2,4-D. However, the lowest regeneration capacity was determined at 2.2% at the dose of 4 mg/l of Ümran Hanım 2,4-D. Our results displayed that auxin type and hormone dosage were very significant on the triticale mature embryos.

Keywords: In vitro culture, Plant regeneration, Callus, Hormones.

Introduction

Increasing plant production is an inevitable reality in order to meet the nutritional needs of the constantly increasing human population. Although many varieties with yield capacity and quality have been developed and brought to the food sector by utilizing traditional plant breeding studies, the desired result has not been fully achieved in resistance to some biotic and abiotic environmental stress, especially diseases and pests. With the advancement of technologies in the field of genetic engineering and the understanding of molecular plant protection mechanisms, new technologies have been developed in the control of stress factors. Cell and tissue culture techniques are a very effective method that uses molecular and cellular-based special technologies to increase the productivity of plant and plant products, or to eliminate the factors

* *Corresponding Author:* Ismail Bezirganoglu,
Department of Molecular Biology and Genetics,
Erzurum Technical University, 25050 Erzurum, Turkey.
E-mail: ismail.bezirganoglu@erzurum.edu.tr

that cause abiotic and biotic stresses, thus preventing plant yield losses [1-4]. In triticale breeding studies, there are intensive studies on agronomic characters such as grain yield, plant height and nutrient content obtained by classical methods, and a good regeneration method should be established from tissue and cell cultures in order to benefit effectively from biotechnological applications used in plant breeding [5]. Therefore, the success of tissue and cell research depends on a sustainable and effective callus culture and plant regeneration process. Immature embryos are generally used as an explant source for somatic callus culture in cereals. Progress has been made in changing the agronomic characteristics of cultivated plants and developing varieties resistant to stress factors, with tissue culture methods that eliminate adverse environmental conditions under controlled conditions developed in recent years [6-8]. Although the success rate in tissue culture is lower in monocotyledons, especially in the Graminea family, than dicotyledons, today it is used in bread wheat [9], maize [10], rice [11] and barley [12] plant regeneration was achieved. There are relatively few studies on the in vitro regeneration of triticale using mature and immature embryos compared to other cereal species. The lack of an effective in-vitro system limits tissue culture studies. The factors that determine the response to tissue culture in cereals, including triticale, are the components of the donor plant, the development status of the explant, and the culture medium [13]. Although there is a successful protocol for plant regeneration using embryos of mature seeds with triticale, there are very few studies on callus formation and regeneration frequency using different plant growth regulators. In many reports on callus regeneration, the most commonly used plant growth regulator to stimulate somatic embryogenesis is 2,4-D (2,4-Dichlorophenoxy acetic acid), picloram (4-amino-3,5,6-trichloropicolinic acid) and dicamba (3,6-Dichlorobenzoic acid) are also used alone or together with 2,4-D [14]. In a study of triticale immature embryo culture, picloram was superior to 2,4-D in embryogenic callus formation and plant regeneration [15]. In another study, it was noted that dicamba provided callus formation faster than 2.4-D and plant regeneration was 2 times higher from the formed calli [16]. In a study of immature embryo culture in wheat, dicamba was found to be better than 2,4-D and picloram in terms of embryogenic callus formation and plant regeneration [17]. Ma and Pulli, 2004 indicated that dicamba was found to be more effective in embryogenesis than 2,4-D and NAA (Naphthalene acetic acid) in immature embryo culture in the rye [18]. The aim of this study is to improve callus formation and plant regeneration efficiency by developing an effective callus formation and the plant regeneration system from mature embryos in triticale and to determine the response of some triticale genotypes to tissue culture of different plant growth regulators and hormone concentrations.

Materials and Methods

Plant material and culture condition: *Ümran Hanım and Melez 2001* genotypes were provided from East Anatolia Agricultural Research Institute. In this report, mature embryos of Ümran Hanım and Mikham 2001 genotypes were used as explants. The mature seeds were mixed in 70% ethanol for 5 minutes and washed 3 times with sterile distilled water. It was then mixed in 1% sodium hypochlorite containing a few drops of Tween 20 (Sigma) for 20 minutes. After surface sterilized seeds were washed with sterile distilled water, they were kept in sterile distilled water at 25°C for 3 hours in the dark. Mature embryos were obtained for callus formation include MS (Murashige and Skoog, ascorbic acid) containing 2 different auxin types (2,4-D, and dicamba), 3 different doses (4.0, 8.0 and 12.0 mg/l), agar (8 g/l) and 1.95 g/l MES. The pH of the medium was adjusted to 5.8 with NaOH (Sodium hydroxide). A factorial experiment was conducted in a

completely randomized design with three replications. Twenty five mature embryos were placed in each petri dish and each petri dish was accepted as an experimental unit. Callus formation (%) [CF% = (Number of callus formed/Explant number) x100], embryogenic callus formation (%) (ECF% = (Embryogenic callus number/ Callus number) x100] was determined.

Results

Callus formation: The callus formation rate ranged from 83.69-93.77% in all application hormone concentrations of two different genotypes used in the experiment (data not shown).

Embryogenic Callus Formation: Calli was evaluated as embryogenic and non-embryogenic calli, whereas embryogenic ones had the potential to form somatic embryos. Callus formation was observed in over 80% of all explants cultured. The difference

Table 1. Embryogenic callus formation ration in terms of callus formation (%)

Hormones	Dose (mg/l)	Genotype		Mean
		Mikham 2001	Ümran Hanım	
2,4-D	4,0	19.3+6.12	14.08+8.06	16.65
	8,0	14.17+6.46	12.86+5.17	13.51
	12,0	33.98+8.02	32.38+9.17	33.18
	Mean	22.48+10.47	19.77+11.11	21.12
Dicamba	4,0	25.04+6.48	39.47+3.92	32.25
	8,0	17.80+2.89	39.45+6.32	28.62
	12,0	35.49+9.33	46.91+7.91	41.2
	Mean	26.11+9.78	41.95+6.85	34.03
Mean		24.30+10.29	30.86+14.47	27.58

between genotypes in terms of embryogenic callus formation rate, according to callus number was found to be statistically significant ($p<0.05$). When the general averages of auxin types and doses are evaluated according to genotypes, it is seen that the rate of callus formation rate is Ümran Hanım 30,863 and Mikham 2001 24,300% (Table 1). While the embryogenic callus formation rate was 19.77% in the 2,4-D application, it was 41.95% in the dicamba application. In Mikham 2001, these rates were 22.48% and 26.11% in media containing 2,4-D and dicamba, respectively. Considering the combination of all the factors used in the experiment, the highest embryogenic callus formation rate (46.91%) was determined at 12 mg/l of Ümran Hanım dicamba. On the other hand, the lowest embryogenic callus formation rate was found with a value of 12.86% in Ümran Hanım's 2,4-D 8.0 mg/l dose (Table 1).

Table 2. Embryogenic callus formation ratio in terms of auxin (%)

Genotypes	Mean (mg/l)			
	4.0 mg/l	8.0 mg/l	12.0 mg/l	Mean
Mikham 2001	22,17	15,98	34,73	24.29
Ümran Hanım	26,77	26,16	39,65	30.86
Mean	24.47	21.07	37.19	27.575

The embryogenic callus formation ratio in terms of 4.0 mg/l, 8.0 mg/l and 12.0 mg/l dose applications, Ümran Hanım was obtained 26.77%, 26.16% and 39.65%, whereas Mikham 2001 was obtained as 22.17%, 15.98% and 34.73%, in the same order (Table 2).

Regeneration Capacity: Embryogenic calli with green plantlets were evaluated as embryogenic calli with regeneration capacity. When the general averages of auxin types and doses were evaluated according to genotypes, the rate of regeneration capacity in terms of the number of callus was 6.33% for Ümran Hanım and 7.15% for Mikham 2001. The rate of regeneration capacity, according to the number of callus in Ümran Hanım was 4.91% in the 2,4-D application and 7.74% in the dicamba application. In Mikham 2001, these rates were 5.54% and 8.76%, respectively, Considering the combination of all the factors used in the experiment, the highest regeneration capacity (12.09%) was determined at a dose of 12.0 mg/l of Mikham 2001 2,4-D. On the other hand, the lowest regeneration capacity (2.2%) was determined at the dose of 4 mg/l of Ümran Hanım 2,4-D (Table 3). Regeneration capacity in terms of 4.0 mg/l, 8.0 mg/l

and 12.0 mg/l dose applications, Ümran Hanım was obtained 3.5%, 6.3% and 9.03%, whereas Mikham 2001 was obtained as 6.7%, 5.3% and 9.3%, in the same order (Table 4).

Table 3. Plant regeneration ratio in terms of callus formation (%)

Hormones	Dose (mg/l)	Genotype		Mean
		Mikham 2001	Ümran Hanım	
2,4-D	4,0	4.72+3.11	2.25+3.32	3.48
	8,0	5.30+3.86	3.72+3.90	4.51
	12,0	6.60+3.87	8.76+6.28	7.68
	Mean	5.54+3.46	4.91+5.21	5.22
Dicamba	4,0	8.71+3.67	4.87+4.46	6.79
	8,0	5.48+3.76	9.05+7.91	7.26
	12,0	12.09+3.49	9.30+6.21	10.69
	Mean	8.76+4.38	7.74+5.06	8.25
Mean		7.15+0.78	6.33+5.24	6.74

Table 4. Plant regeneration ratio in terms of auxin doses (%)

Genotypes	Dose (mg/l)			
	4.0 mg/l	8.0 mg/l	12.0 mg/l	Mean
Mikham 2001	6,72	5,30	9,34	7.15
Ümran Hanım	3,56	6,39	9,03	6.33
Mean	5.14	5.89	9.19	6.74

Discussion

Success in callus formation and plant regeneration studies in many plant species, including cereals largely depends on genotype, explant type and media components. Genotype is the most important factor affecting the somatic embryogenesis of cereals from mature (19, 20, 5). In this study, it was determined that there were significant differences between the genotypes used on embryogenic callus formation and the number of regenerated plantlets. In similar studies on this subject, it has been reported that the effect of genotype on embryogenic callus formation is very important, according to callus number [21, 22]. These findings are in agreement with those published by Rakoczy-Trajanonowska and Malepszy [23] on rye in the study of the rate of embryogenic callus formation varies according to genotype. Several reporters observed that 2 weeks after callus formation in inbred lines of rye, yellow, hard and nodular calli were formed on 2 lines, and white and watery embryogenic calli were formed in the remaining 5 lines. In a study by Aydın et al. [14] in which they investigated the factors affecting tissue culture using embryos matured in wheat, they determined that the effect of genotype on the number of somatic embryos was important and that dicamba was more effective than 2.4-D and picloram as an auxin type. The concentration of plant growth regulators in the culture medium has an important effect on morphogenesis and growth. Generally, high concentration of auxins and low cytokinin increase callus formation and cell proliferation. Regardless of the explant source, 2,4-D triticale is the most widely used plant growth regulator for callus formation and maintenance. The results of this study determined that the frequency of callus formation and embryogenic callus formation was much higher in the medium containing dicamba. Unlike the findings, we obtained in this study, Vikrant and Rashid [22] stated that low concentrations of plant growth regulators are more effective in forming somatic embryos. Zapata et al., [24] stated that non-high doses gave better results in plant regeneration from embryogenic calluses of barley. Venkant et al., [25] observed that embryogenic calli regenerated by forming somatic embryos in a dose of 2.0 mg/l and within 3-4 weeks. Considering the combination of all the factors used in the experiment, when a comparison is made between auxin types, it is seen that dicamba is in the first place in terms of regeneration capacity. Papenfus and Carman [26] determined that dicamba was more effective than 2,4-D on regeneration capacity in immature embryo culture in wheat. Satyavathi et al., [17] reported that dicamba was more effective than 2,4-D and picloram in plant regeneration as well as in callus formation. Similarly, Mendoza and Kappler [27] found that the number of plants per embryo was 2 times higher in the

medium containing dicamba in mature embryo culture in wheat. Zimny and Lörz [13] tested immature embryos in rye with different doses of different auxins for MS [28], N6, CC, and B5 in four different basic media to determine somatic embryogenesis and plant regeneration, and CC containing 30 µM dicamba bested obtained in the environment. Similar to the results mentioned above, it was concluded that dicamba is more effective than 2,4-D in terms of embryogenic callus formation and plant regeneration, but our results show that high-dose applications rather than low doses of dicamba play an active role in creating higher regeneration. Mature embryos of Ümran Hanım and Mikham 2001 genotypes of triticale were tested in MS medium with different plant growth regulators and at different doses in order to determine callus, embryogenic callus formation and regeneration capacity. Callus formation rate, embryogenic callus formation according to callus number, regeneration capacity rates, according to callus number was investigated. According to the results obtained from this study, the effect of genotype, plant growth regulators and dose on callus formation rate was observed, and statistically significant differences were found between genotypes in terms of embryogenic callus formation rate.

Regeneration capacity, genotype, plant growth regulators and dose applications were effective, according to callus number and embryogenic callus number. According to the findings obtained from the study, it can be recommended to use high doses of 12.0 mg/l dose of Ümran Hanım genotype and dicamba, a plant growth regulator, in studies on the mature embryo culture of triticale.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: İB, SK, BR, FB and BY contributed to the study conception and design. SK, BR, FB and BY contribution to laboratory work. Literature research (İB and BY), Writing the article (İB and BR). All authors read and approved the final manuscript. İB: İsmail Bezirganoğlu, SK: Serap Kahraman, BR: Büşra Reisoğlu, FB: Fatma Böke, BY: Büşra Yazıcılar.

ORCID

Ismail Bezirganoglu 0000-0003-4079-5998
Serap Karaman 0000-0003-1216-0012
Beyza Reisoğlu 0000-0003-1149-9022
Fatma Böke 0000-0002-4543-6575
Büşra Yazıcılar 0000-0003-2465-7579

References

1- Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, et al.. Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 1997;3:87-103.
2- Oldah HK, Becker D, Lörz H. Heterologous Expression of Genes Mediating Enhanced Fungal Resistance in Transgenic Wheat, Molecular Plant-Microbe Interactions 2001;7:832-838.
3- Rajamat MV, Kumria R, Singh S. Molecular biology and genetic engineering in plants. Pp: 60-77, In: Plant Biotechnology and Molecular Markers 2006.
4- Şehirali S, Özgen M. Bitki Islahı. Ankara Üniv Zir Fak Yay 2007;1553/506, 270 sayfa, Ankara.
5- Ulukan H. Tahıllarda In vitro Çalışmalar, Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi 2005;8:19–31.
6- Deo PC, Taylor M, Harding RM, Tyagi AP, Becker DK. Initiation of embryogenic cell suspension of taro (Colocasia esculenta) and plant regeneration. Plant Cell Tissue Organ Cult 2010; 100:283-291.
7- Mahipal S, Monokari M. Somatic embryogenesis and in vitro flowering in Hybanthus enneaspermus (L.) F. Muell. – A rare multipotent herb. Asian Pacific Journal of Reproduction 2016;5(3):256-262.
8- Pence VC. In vitro methods and the challenge of exceptional species for Target 8 of the Global Strategy for Plant Conservation. Ann Missouri Bot Gard 2013;99:214-220.
9- Redway FA, Vasil V, Lu D, Vasil IK. Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (Triticum aestivum L.). Theor Appl Genet 1990;79: 609–617.
10- Duncan DR, Williams ME, Zehr BE, Widholm JM. The production of callus capable for plant regeneration from immature embryos of numerous Zea mays genotypes. Planta 1985;165:322-332.
11- Yamada Y, Yang ZG, Tang DT. Plant regeneration from protoplast- derived callus of rice (Oryza sativa L.). Plant Cell Reports 1986;5:85-88
12- Luhrs R, Lorz H. Plant regeneration in vitro from embryogenic cultures of spring and winter type barley (Hordeum vulgare L.) varieties. Theor Appl Genet 1987;75:16-25.
13- Zimny J, Lörz H. High frequency of somatic embryogenesis and plant regeneration of rye (Secale Cereale L.). Plant Breeding 1989; 102:89-100.
14- Aydın M, Sağsöz S, Haliloğlu K, Tosun M. Buğdayda olgun embriyo kültürünü etkileyen faktörler. Atatürk Üniv. Ziraat Fak Derg 2011;42 (1):1-10.
15- Przetakiewicz A, Orczyk W, Nadolska-Orczyk A. The effect of auxin on plant regeneration of wheat, barley and triticale. Plant Cell, Tissue and Organ Culture 2003;73: 245–256.
16- Ainsley PJ, Aryan AP. Efficient plant regeneration system for immature embryos of Triticale Triticosecale Wittmack). Plant Growth Regul 1998;24:23–30.
17- Satyavathi VV, Jauhar Elias EM, Rao MB. Effects of growth regulators on in vitro plant regeneration in durum wheat. Crop Science, 2004;44:1830-1846.

18- Ma R, Pulli S. Factors influencing somatic embriyogenesis and regneration ability in somatic culture of spring and winter rye. Agriculture and Food Science, 2004;13:363- 377.
19- He GY, Lazzeri AP. Improvement of somatic embriyogenesis and plant regeneration from durum wheat (Triticum turgidum var. durum Desf.) scutellum and inflorescence cultures. Euphytica, 2001;119:369-376.
20- Tsugawa H, Suzuki M. A low-temperature method for maintaining plant regeneration activity in embryogenic callus of rice (Oryza sativa L.). Plant Cell Reports 2000;19:371-375.
21- Fennel S, Bohorova N, Ginkel M, Crossa J, Hoisington DA. Plant regeneration from immature embryos of 48 elite CIMMYT bread wheats. Theor Appl Genet 1996;92:163– 169.
22- Vikrant, Rashid A. Direct as well as indirect somatic embryogenesis from immature (unemerged) inflorescence of a minor millet Paspalum scrobiculatum L. Euphytica, 2001; 120:167-172.
23- Rakoczy-Trojanowska M, Malepszy S. Genetic factors influencing regeneration ability in rye (Secale cereale L.). II. Immature embryos. Euphytica 1995;83:233–239.
24- Zapata JM, Sabater B, Martin M. Callus induction and in vitro regeneration from barley mature embryos. Biol Plant, 2004;48(3):473–476.
25- Venkant RB, Jauhar PP. Regeneration of plantlets through isolated scutellum culture of durum wheat. Plant Science1996;197-203.
26- Papenfuss JM, Carman JG. Enhanced regeneration from wheat callus cultures using dicamba and kinetin. Crop Science, 1987;27:588–593.
27- Mendoza MG, Kaeppler HF. Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of Wheat (Triticum aestivum). In vitro Cell Dev Biol-plant 2002;38:39-45.
28- Murashige T, Skoog F. A revised medium for rapid growth and bioassays tobacco cultures. Physiol Plant. 1962;15:473–497.

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

The Effects of Quercetin Administration on Heart Tissue and Serum Parameters in the Rats with Experimental Obesity

Cihan GÜR[1], Seçkin ÖZKANLAR[1], Semin GEDİKLİ[2], Emin ŞENGÜL[3], Volkan GELEN[4*], Adem KARA[5]

[1]*Ataturk University, Veterinary Faculty, Department of Biochemistry, 25240, Erzurum /Turkey*

[2]*Ataturk University, Veterinary Faculty, Department of Histology and Embryology, 25240, Erzurum/ Turkey*

[3]*Ataturk University, Veterinary Faculty, Department of Physiology, 25240, Erzurum/ Turkey*

[*4]*Kafkas University, Veterinary Faculty, Department of Physiology, 36100, Kars/ Turkey*

[2]*Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 25050, Erzurum/ Turkey*

Cite: *Gür C, Özkanlar S, Gedikli S, Şengül E, Gelen V, Kara A. The Effects of Quercetin Administration on Heart Tissue and Serum Parameters in the Rats with Experimental Obesity. Eurasian Mol Biochem Sci 2022;1(1): 16-21.*

Received: 16 March 2022, Accepted: 04 April 2022 *DOI. 10.54672/ejmbs.2022.316.5*

Abstract

Obesity causes structural and functional damage to various organs. Quercetin is a flavonoid and has potent antioxidant, anti-apoptotic and anti-inflammatory effects in the body cells. This study aims to investigate the therapeutic effect of Quercetin on the cardiac effect caused by obesity in rats with experimental obesity using biochemical and histological methods. In this study, 24 male Sprague Dawley rats were used. The rats were divided into three groups i.e., control, obese, and Quercetin-Obese. A high-fat diet was administrated to the obese groups for 3 months, the other groups were fed with normal pellet forage. After the formation of obesity, a 50 mg/kg dose of Quercetin was orally fed to the quercetin-obese and quercetin groups for 15 days. At the end of study, all the animals were sacrificed by taking blood under anesthesia (sevoflurane), and their heart tissues were taken. In the obtained serum samples, the levels of triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and cardiac troponin-I (cTnI) were measured by the auto-analyzer device. The heart tissues were stained with Bax and Bcl-2 antibodies as immuno-histochemical. When the results of the analysis were compared among the control and other groups, it was shown that obesity increased the levels of serum TG, CHOL, LDL-C, and cTnI whereas quercetin administration had a decreasing effect on these parameters ($P<0.05$). It was determined that the level of Serum HDL-C decreased in the obese group while quercetin administration increased the level of HDL-C ($P<0.05$). The analysis of Bax and Bcl-2 immune reactive cells showed that the apoptotic cell density increased in the heart tissues of the obese group while quercetin administration decreased the apoptotic cell density ($P<0.05$). This study shows that Quercetin may have a therapeutic effect in avoiding cardiac injuries caused by obesity.

Keywords: Obesity, Quercetin, Heart, Cholesterol, Apoptosis

* *Corresponding Author:* Volkan GELEN,
Kafkas University, Veterinary Faculty, Department of Physiology,
36100, Kars/ Turkey
E-mail: gelen_volkan@hotmail.com

Introduction

Obesity is defined as the excess of fat tissues or an increase in body mass index, which is described as a paradox that started in the 1980s and became an epidemic worldwide. Obesity can cause various complications such as heart failure, coronary heart disease, type 2 diabetes mellitus, and hypertension in humans [1,2]. One of the most significant risk factors of obesity is heart failure and is one of the biggest life-threatening health problems [3]. Previous studies showed that obesity causes heart failure due to myocardial damage, but the exact mechanism of cardiac injury is not fully understood [4]. It has been reported that the high levels of LDL-C and triglyceride values and low levels of HDL-C play an essential role in the formation of damage [5]. Troponin-I is a cardiac-specific protein used as a specific marker in measuring the extent of the injury [6]. To diagnose heart damage or to determine whether the clinical signs are due to cardiac causes, researchers have recently recommended high-sensitivity measurements of cardiac troponin-I in cardiac injury [7].

Quercetin is a flavonoid and has potent antioxidant, anti-apoptotic and anti-inflammatory effects in the body cells [8]. Quercetin is one of the most abundant dietary flavonoids with extensive pharmacological and antioxidant properties [9]. One of the pharmacological properties of Quercetin is cardiac protection [10]. Among the essential cardiac protective properties are anti-hypertensive and anti-atherogenic effects. In addition, it prevents endothelial dysfunction and protects the myocardium from ischemia [11]. It is believed that its antioxidant property comes from its ability to chelate Fe2+ and Cu2+ ions and its scavenging feature of free radicals [12]. Besides, previous studies have revealed the protective role of inhibiting oxidative damage [13]. For this reason, this study aimed to investigate the cardiac effects of experimental obesity in rats and the role of Quercetin in the prevention of obesity-mediated cardiac damage by biochemical and histological methods.

Materials and Methods

Animal housing and procedure: This study used 24 adult male Sprague Dawley rats, 10-12 weeks old (average weight of 200-250g), obtained from Atatürk University Medical Experimental Research and Application Center. The study was approved by Atatürk University Experimental Animals Ethics Committee (Decision No: 2022/52). The rats were kept in cages at 25 ± 2 °C temperature, 60-65% humidity with 12-hour light and 12-hour dark cycles, fed *ad libitum*. The cages were cleaned daily and after one week of acclimation, rats were randomly divided into four groups Control, Obesity, Quercetin-Obesity, and Quercetin.

Obesity induction: The rats were fed a high-fat diet (35 kcal% as fat) for the experimental model of obesity for 120 days as reported in previous studies [14]. Control group animals were fed with a normal ad libitum diet (10% kcal as fat). Bodyweight gain was measured at the beginning of study and weekly reviewed for obesity assessment, along with food consumption. Rats that reached 8-20% weight gain according to the initial weight determined as the obesity criterion were accepted as obese. At the end of study, non-obese rats were excluded from the study.

Quercetin treatment: In the period following the onset of obesity (120 days later), quercetin and quercetin-obese groups were administered orally at 50 mg/kg suspended Quercetin in 1 ml corn oil for 15 days. The obese group was given 1 ml of corn oil orally for 15 days. At the end of the study, cardiac blood samples were taken from all groups of rats under deep anesthesia with sevoflurane (3%) and then were sacrificed and their heart tissues were removed for histological examination.

Biochemical analysis: To obtain sera samples, the blood samples of rats were centrifuged at 1700 g for 8

min. The serum samples were stored in the freezer at –80 °C before they were serum enzyme analyses. The serum samples were measured for the levels of triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and cardiac troponin-I (cTnI) by using the auto-analyzer (Beckman Coulter; DXI 800 USA).

Histologic analysis: Obtained heart tissues were fixed in 10% neutral formaldehyde for 72 hours and then washed with tap water and overnighted. The tissues were passed from the ascending alcohol series and cleaned with xylene. The tissues were then embedded in paraffin blocks and 5 µm thick tissue cuts were stained with Crossman modified Mallory's Triple staining. The tissue sections were examined in a trinocular light microscope (Nikon Eclipse 50i, Japan).

Immunohistochemical staining: After deparaffinized and dehydrating, the sections were left in 3% hydrogen peroxide phosphate buffer solution (PBS) at pH 7.4 for 20 minutes. Sections passed through PBS were left in sodium citrate buffer (pH 6.0) in a 600 W microwave oven to reveal antigenic receptors. Sections were incubated in Blocking Solution A (Invitrogen-Histostatin Plus Bulk Kit) for 8 min. Then Bax (1/50 dilution, Abcam) and Bcl-2 (1/50 dilution, Abcam) primary antibodies (Abcam, Ab183855) (1:500) were treated in sections for 1 hour at 37 C^{o} in the humidity chamber. After the sections were left for 30 min in biotin secondary antibody, Blocking Solution C (streptavidin peroxidase) was added and they were gone for another 30 min. Then diaminobenzidine (DAB) chromogen solution was added and hematoxylin staining was applied for contrast staining. Immunohistochemical examinations were performed under a light microscope (Nikon Eclipse 50i, Japan) and then photographs were taken. Semiquantitative analyses for all groups investigated the Bax and Bcl-2 immunoreactivity degree at the cellular level. To estimate the immune reactive cell count, staining intensity was measured using the stereological optical fractionator method, as described in detail in our previous studies [15,16].

Statistical analysis: The statistical significance among groups was analyzed by one-way ANOVA (Duncan *post hoc* test). The results were considered statistically significant when $p < 0.05$. Data are expressed as mean ± Standard deviation (SD).

Results

Biochemical results: Serum TG, CHOL, LDL-C, and cTnI levels were increased in the obesity group compared with the Control group ($p<0.05$), On the other hand, these parameters were decreased in the Obesity-Quercetin group compared with the Obesity group ($p<0.05$). While it was determined that the serum HDL-C level decreased in the obese group, quercetin administration significantly increased the HDL-C level ($P<0.05$).

Histologic analysis: Two specialists in histology blindly evaluated the possible cardiac damages. The lesions have been assessed as shown below. In the case

Table 1. Serum triglyceride (TG) cholesterol (CHOL), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and Cardiac Troponin-I (cTnI) levels for all groups.

	TG	CHOL	HDL-C	LDL-C	cTnI
Control	69.24±16.32^{a}	61.80±17.39^{a}	45.33±9.28^{a}	20.17±3.64^{a}	6.28±1.75^{a}
Obesity	83.86±17.44^{b}	77.75±15.38^{b}	55.82±12.58^{b}	27.44±6.85^{b}	12.38±1.86^{b}
Quercetin-Obesity	72.54±15.53^{a}	70.33±13.32^{a}	48.10±13.8^{a}	21.75±7.42^{a}	7.72±2.62^{a}

The letters indicated the statistical differences in the column, P-value was accepted as 0.05. Data were analyzed with ANOVA (Duncan) test.

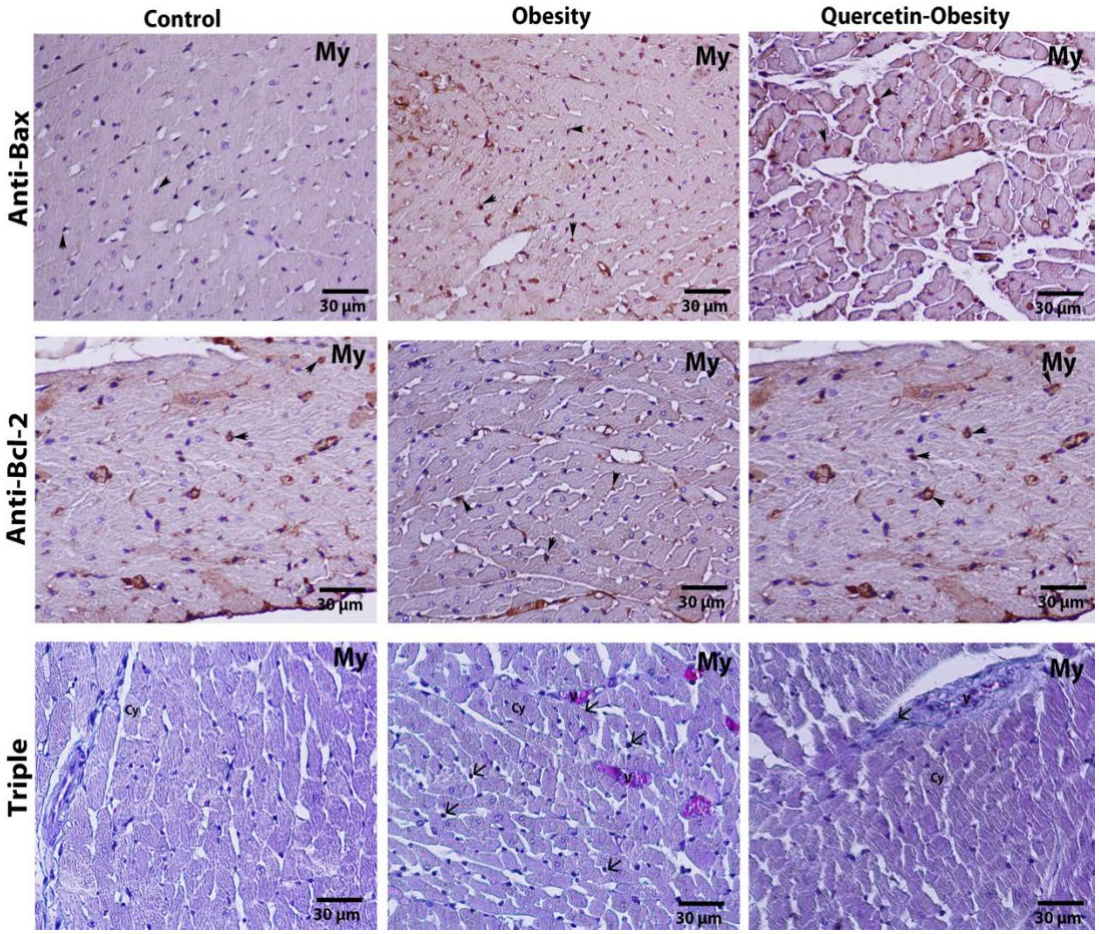


Figure 1. Illustration of anti-Bax, anti-Bcl-2, and Crossman Modified Triple staining in the heart tissues for all groups, My: myocardium, Cy: transverse area of cardiomyocyte, **arrowhead:** anti-Bax and anti-Bcl-2 immune reactive cells, **open arrows:** PNML cells

of the study of cardiomyopathy, inflammation and fibrosis have been assessed. In the examination of the myocardium and subendocardial fibrosis. On the other hand, these changes were considerably decreased in Quercetin- Obesity group (Figure 1).

Stereological Immunohistochemical analysis: In the stereological analysis of anti-Bax and anti-Bcl-2 immune reactive cells, Bax immune reactive cell density was increased in the Obesity group while decreased in the Quercetin-Obesity group ($p<0.05$). Besides, Bcl-2 cell density was reduced in the Obesity group compared with the Control group. However, it increased in the Quercetin-Obesity group ($p<0.05$). The anti-Bax and anti-Bcl-2 immune reactive cell densities and comparisons are seen in Table 2.

Table 2. Stereological evaluation of anti-Bax and anti-Bcl-2 positive cells densities in the serially obtained heart tissue sections per 1000 μm^2 area.

Groups	Bax	Bcl-2
Control	1,24±0,33[a]	3,80±0,79[a]
Obesity	3,86±0,94[b]	1,75±0,38[b]
Quercetin-Obesity	2,54±0,53[c]	2,33±0,56[c]

The letters indicated the statistical differences in the column, P-value was accepted as 0.05. Data were analyzed with ANOVA (Duncan) test.

Discussion

Quercetin is a flavonoid and its effects are currently being investigated in the prevention of many tissue or organ damages [17]. On the other hand, its anti-obese and anti-apoptotic effects have been shown in previous studies [18-21]. In the present study, a metabolic

obesity rat model was created, and Quercetin was administered at a dose of 50mg/kg which is in line with previous studies [22].

The high-fat diet used in the present study was sufficient intensity and duration to induce obesity in rats. Also, previous research suggested that rats fed with a high-fat diet for eight weeks caused obesity and impaired hypertension, inflammation, dyslipidemia, endothelial dysfunction, and cardiac fibrosis in the heart [23,24]. These changes were closely similar to the human metabolic syndrome. In the present study, Serum TG, CHOL, LDL-C, and Trop-I levels were increased and serum HDL-C level was decreased in the obesity group. But Quercetin treatment reduced TG, CHOL, LDL-C, and cTnI levels and increased the serum HDL-C level in the obese rats.

In histopathologic evaluation, eosinophilic changes, inflammatory cell infiltration, and fibrosis were observed in the obesity group heart tissues. A previous study reported the interstitial collagen deposition in the left ventricle of the obesity group [25]. However, other studies reported that did not find a histological increase in cardiac collagen in a rat model of diet-induced obesity [26] in rabbits [27]. Possible mechanisms of collagen deposition are related to inflammation and cell infiltration. Additionally, it has been reported that the higher collagen deposition is related to abnormalities in insulin metabolism [25]. Quercetin has an anti-inflammatory effect that inhibited inflammation-mediated collagen deposition and cardiac damage in obesity [28].

Increased apoptotic activity was determined in the heart tissue in the case of obesity. Bax and Bcl-2 proteins are apoptosis-related proteins that determine cell death. It has been reported that obesity-induced apoptosis is shaped by lipid accumulation in the endoplasmic reticulum in the cell [29]. In this study, the levels of the proapoptotic member of the Bcl-2 family and the anti-apoptotic member of Bax were investigated by immunohistochemical analysis. The results showed that while the Bax level increased in the obesity group, it decreased in the quercetin-obesity group. It was determined that the Bcl-2 level decreased in the obesity group and raised in the quercetin-obesity group. Similar to us, previous studies reported the cardioprotective effects of Quercetin in high-fat diet-induced obesity [28,30, 31].

In conclusion, the present data provide scientific evidence that Quercetin may provide protection against cardiac damage and may reduce lipid accumulation and inflammation in the heart tissue. Quercetin contained multiple bioactive compounds which might act synergistically to produce protective effects.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: CG, SÖ, SG, EŞ, VG and AK contributed to the study conception and design. CG, VG, AK contribution to laboratory work. Writing the article (SÖ, EŞ, SG and AK). All authors read and approved the final manuscript. CG: Cihan Gür, SÖ:Seçkin Özkanlar, SG: Semin Gedikli, EŞ: Emin Şengül, VG: Volkan Gelen, AK: Adem Kara.

ORCID

Cihan GÜR 0000-0001-6775-7858
Seçkin ÖZKANLAR 0000-0001-7717-797X
Semin GEDİKLİ 0000-0001-8238-7226
Emin ŞENGÜL 0000-0003-1566-1816
Volkan GELEN 0000-0002-5091-1262
Adem KARA 0000-0002-5766-6116

References

1- Lavie CJ, Alpert MA, Arena R, Mehra MR, Milani RV, Ventura HO. Impact of obesity and the obesity paradox on prevalence and prognosis in heart failure. JACC: Heart Failure 2013;1:93-102.
2- Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.
3- Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, et al. Obesity and the risk of heart failure. NEJM 2002;347:305-313.
4- Ndumele CE, Coresh J, Lazo M, Hoogeveen RC, Blumenthal RS, Folsom AR, et al. Obesity, subclinical myocardial injury, and incident heart failure. JACC: Heart Failure 2014;2:600-607.

5- Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. N Engl J Med 1999;341:410-418.
6- Boccara G, Pouzeratte Y, Troncin R, Bonardet A, Boularan AM, Colson P, et al. The risk of cardiac injury during laparoscopic fundoplication: cardiac troponin I and ECG study Note. Acta Anaesthesiol Scand 2000;44:398-402.
7- Adams JE, Sicard GA, Allen BT, Bridwell KH, Lenke LG, Davila-Roman VG, et al. Diagnosis of perioperative myocardial infarction with measurement of cardiac troponin I. N Engl J Med 1994;330:670-674.
8- Sözüer EC, Tarladaçalişir YT. Quercetin Ameliorates the Streptozotocin-Induced Diabetic Renal Injury by Inhibiting Apoptosis. Trak Univ J Nat Sci 2021;22:131-138.
9- Panda S, Kar A. Combined effects of vincristine and Quercetin in reducing isoproterenol-induced cardiac necrosis in rats. Cardiovascular Tox 2015;15:291-299.
10- Perez-Vizcaino F, Duarte J. Flavonols and cardiovascular disease. Mol Aspects Med 2010;31:478-494.
11- Panda S, Kar A. Combined effects of vincristine and Quercetin in reducing isoproterenol-induced cardiac necrosis in rats. Cardiovascular tox 2015;15:291-299.
12- Sestili P, Guidarelli A, Dachà M, Cantoni O. Quercetin prevents DNA single-strand breakage and cytotoxicity caused by tert-butyl hydroperoxide: free radical scavenging versus iron-chelating mechanism. Free Radic Biol Med 1998;25:196-200.
13- Lin CF, Kuo YT, Chen TY, Chien CT. Quercetin-Rich Guava (Psidium guajava) juice in combination with trehalose reduces autophagy, apoptosis, and pyroptosis formation in the kidney and pancreas of type ıı diabetic rats. Molecule 2016;21:334.
14- Gelen V, Şengül E, Gedikli S, Gür C, Özkanlar S. Therapeutic effect of Quercetin on renal function and tissue damage in the obesity-induced rats. Biomed Pharmacother 2017;100: 524-528.
15- Arabacı T, Kermen E, Özkanlar S, Köse O, Kara A. Kızıldağ A, et al. Therapeutic effects of melatonin on alveolar bone resorption after experimental periodontitis in rats: A biochemical and immunohistochemical study. J Periodontol 2015;86:874–881.
16- Akman S, Canakci V, Kara A, Tozoglu U, Arabaci T, Dagsuyu IM. Therapeutic effects of alpha-lipoic acid and vitamin C on alveolar bone resorption after experimental periodontitis in rats: A biochemical, histochemical, and stereologic study. J Periodontol 2013;84:666–674.
17- Rivera L, Morón R, Sánchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity 2008;16:2081-2087.
18- Erboga M, Aktas C, Erboga ZF, Donmez YB, Gurel A. Quercetin ameliorates methotrexate-induced renal damage, apoptosis, and oxidative stress in rats. Renal failure 2015;37:1492-1497.
19- Sengul E, Gelen V, Gedikli S. Cardioprotective activities of quercetin and rutin in Sprague Dawley rats treated with 5-fluorouracil. JAPS 2021;31:423-431.
20- Gelen V, Şengül E, Çınar DA. The effects of rutin and quercetin on ECG parameters in 5-FU-induced cardiotoxicity rat model. WJARR 2021;9:253-257.
21- Sengul E, Gelen V, Gedikli S, Ozkanlar S, Gur C, Çelebi F. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in mice. Biomed Pharmacother 2017;92:303-7.
22- Levin BE, Triscari H, Sullivan AC. Altered sympathetic activity during development of diet-induced obesity in rat. Am J Physiol Regul Integr Comp Physiol 1983; *244*: R347-R355.
23- Panchal SK, Poudyal H, Arumugam TV, Brown L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. The J Nut 2011;141:1062-1069.
24- Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate–, high fat–fed rats. The J Nut 2010;140:946-953.
25- Leopoldo AS, Sugizaki MM, Lima-Leopoldo AP, do Nascimento AF, Luvizotto RDAM, de Campos DHS, et al. Cardiac remodeling in a rat model of diet-induced obesity. Can J Cardiol 2010;26:423-429.
26- Carroll JF, Zenebe WJ, Strange TB. Cardiovascular function in a rat model of diet-induced obesity. Hypertension 2006;48:65-72.
27- Carroll JF, Tyagi SC. Extracellular matrix remodeling in the heart of the homocysteinemic obese rabbit. Am J Hypertens 2005;18:692-698.
28- Sato S, Mukai Y. Modulation of Chronic Inflammation by Quercetin: The Beneficial Effects on Obesity. J Inflamm Res 2020;13:421–431.
29- Ozcan U, Cao Q, Yilmaz E, Lee AH. Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457-461.
30- Ahn J, Lee H, Kim S, Park J, Ha T. The anti-obesity effect of Quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem Biophys Res Commun 2008;373:545-549.
31- Gedikli S, Ozkanlar S, Gur C, Sengul E and Gelen V. Preventive effects of quercetin on liver damages in high-fat diet-induced obesity. *J Histol Histopathol* 2017; 4:7.

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

Antimicrobial and Antibiofilm Activity of Methanol and Ethyl Acetate Extract of *Ferula* sp. Growing in Erzurum

Şimal Topçu[1], Elif Arslan[1,2], Şeymanur Çobanoğlu[1,2], Ayşenur Yazıcı[1,2*], Ümit İncekara[3]

[1]*Erzurum Technical University, Faculty of Science, Molecular Biology and Genetics Department, Erzurum, Turkey.*

[2]*Erzurum Technical University, High Technology Research and Application Centre (YUTAM), Molecular Microbiology Laboratory, Erzurum, Turkey*

[3]*Ataturk University, Faculty of Science, Biology Department, Erzurum, Turkey*

Received: 14 March 2022, Accepted: 12 April 2022 *DOI. 10.54672/ejmbs.2022.4*

Cite: *Topçu Ş, Arslan E, Çobanoğlu Ş, Yazıcı A, İncekara Ü. Antimicrobial and Antibiofilm Activity of Methanol and Ethyl Acetate Extract of Ferula sp. Growing in Erzurum. Eurasian Mol Biochem Sci 2022;1(1): 22-27.*

Abstract

This study investigated the antimicrobial and antibiofilm activities of Ferula species (sp.) obtained from Erzurum province. Antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Candida albicans, and Candida tropicalis were determined by agar well diffusion assay. Ethyl acetate extract showed antimicrobial activity against S. aureus, and methanol extract showed antimicrobial activity against E. coli and C. albicans with a zone diameter of 21, 18, and 15 mm, respectively. The extract's minimum inhibitory concentrations (MIC) were 16, 32, and 32 µg/mL ml for S. aureus, E. coli, and C. albicans, respectively. Furthermore, it was determined for the first time that Ferula sp. extracts were effective against biofilm formations of S. aureus and C. albicans. In the light of these results, we think that extracts of Ferula sp. contain potential candidate molecules against biofilm-associated infections, and further characterization studies are needed.

Keywords: Antimicrobial, Antibiofilm, Extraction, *Ferula* sp.

Introduction

Nowadays, antibiotic resistance has become one of the biggest and most significant problems to human health. One of the most important reasons for antibiotic resistance is the formation of biofilms by microorganisms. Biofilms are cell communities composed of an extracellular matrix of polysaccharides, proteins, and DNA (1). Biofilms are highly resistant to antimicrobial molecules. There is a need for new antimicrobial and antibiofilm compounds in the fight against infections caused by biofilm structure and multi-drug resistant bacteria, which are

* *Corresponding Author:* Ayşenur Yazıcı,
Erzurum Technical University, Faculty of Science,
Department of Molecular Biology and Genetics, 25050, Erzurum, Turkey,
E-mail: aysenur.ozdemir@erzurum.edu.tr

a severe problem worldwide. Therefore, the discovery of antimicrobial sources becomes a necessity.

Plants are potential candidates for natural products to help combat such problems. There are plants with antimicrobial, antibacterial, antifungal, and antioxidant activity (2,3,4). So far, more than 30,000 compounds have been isolated from these plants (5). In addition, plant extracts with antimicrobial activity are used in many biotechnological applications. Compounds from plants that are effective on pathogens and have low toxicity for humans are essential in developing new antimicrobial drugs (6,7).

Ferula sp. belonging to the Apiaceae family have been identified as a rich source of antimicrobial compounds. It contains more than 150 species, mainly in the Mediterranean and Central Asia. For hundreds of years, products from Ferula sp. have been used in traditional medicine for skin infections, diarrhea, killing intestinal parasites, malaria, and microbial diseases (8,9,10,11). Ferula sp. in Turkey are called çakşir, çakşir grass or çaşir. Ferula L., the third largest genus of the Apiaceae family, is 18 in the flora of Turkey, only 9 of which are endemic (12,13). These species contain rich secondary metabolites and antimicrobial phytochemicals such as tannins, alkaloids, terpenoids, flavonoids, and coumarins (13). Studies show that it protects against bacteria and fungi that cause food contamination.

The current study evaluated Ferula sp. obtained from Erzurum province and Tortum town for antimicrobial and antibiofilm activity against pathogenic bacterial and fungal microorganisms.

Materials and Methods

Plant, Microbial Strains and Growth Conditions: *Ferula* species were obtained from Erzurum province and Tortum town in this study. *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212) bacteria, and *C. albicans* (ATCC 10231) and *C. tropicalis* (KUEN 1025) yeasts were used in this study. In a shaking incubator, bacterial cultures were incubated in Mueller Hinton broth (MHB) for 24 hours at 37°C and 120 rpm. In a shaking incubator, yeast cultures were incubated in Potato dextrose broth (PDB) for 24 hours at 30°C and 120 rpm.

Extraction of *Ferula* Sp.: The plants were washed in a 0.04% bleach and then rinsed with distilled water. The plants were divided into sections by weighing 10 g as stems and leaves. These sections were homogenized separately with ethyl acetate and methanol. The shredded samples were taken and incubated in a magnetic shaker at 500 rpm for 2 hours. At the end of the period, the solid particles were separated by filtration and evaporated in the rotary evaporator. The resulting substantial extracts were used for further studies and dissolved with 1% dimethyl sulfoxide (DMSO) before use (14).

Agar Well Diffusion Assay: The agar well diffusion test was used to qualitatively assess the efficacy of antimicrobial activity of *Ferula* sp. Three wells were drilled in each petri dish with a cork borer. 150 µL ml *Ferula* sp. extracts were placed in the wells of agar plates inoculated with the test microorganisms. Petri dishes were incubated at 37°C for 24 hours in a flat position. At the end of the period, the zone diameters were measured. DMSO (1%) was used as a negative control (15).

Microdilution Assay: The microdilution assay was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (16). Briefly, 96-well plates were used to determine the minimum inhibitory concentrations (MIC) of the extracts. 100 µl of bacteria and yeast cells, the concentration of which was adjusted to OD_{600} =0.08-0.1, was added to each well, and the extract was added to the wells in the range of 1-128 µg/mL ml. The total volume was brought to 200 µl. The group with no cells

was added as a negative control, and the group with no extract was added as a positive control. Microorganism growth in the wells was evaluated after 24 hours of incubation at 37 °C. The MIC value was determined as the lowest concentration without visual growth.

Crystal Violet Assay: To evaluate the antibiofilm activities of *S. aureus* (ATCC 25923) and *C. albicans* (ATCC 10231), 96-well plates were cultivated similarly to the microdilution assay. These plates were incubated at 37 °C for 48 hours. After that, all the contents in the wells were discarded and washed three times with phosphate buffer. 200 µl of 0.1% crystal violet dye was added and the plates were incubated in the dark for 20 minutes. Then, the wells were washed and were fixed with 30% acetic acid. An absorbance measurement was taken at 590 nm. The evaluation was made according to negative and positive control (17).

Results

Ethyl acetate and methanol extracts of *Ferula* sp. showed antimicrobial activity against *S. aureus, C. albicans,* and *E. coli*. However, the best results were determined as ethyl acetate extract against *S. aureus* shown in Figure 1 and methanol extract against *E. coli* and *C. albicans*. The zone diameters measured are represented in Table 1. Neither extract showed antimicrobial activity against other pathogenic bacteria.

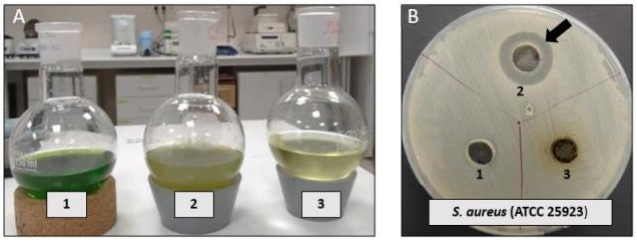


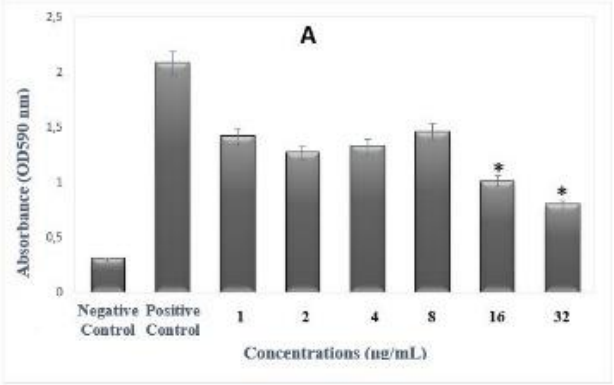
Figure 1. A. Extraction flask of *Ferula* sp. (1) Methanol extract of *Ferula* sp. leaf. (2) Ethyl acetate extract of *Ferula* sp. root. (3) Methanol extract of *Ferula* sp. root. B. Antimicrobial activity of ethyl acetate extract against *S. aureus*.

MIC values of *Ferula* sp. extract were 16, 32 and 32 µg/mL ml against *S. aureus*, *E. coli* and *C. albicans*, respectively (Table 1).

S. aureus and *C. albicans* were selected for antibiofilm activity studies, where we had the best results. The ethyl acetate extract of the collected *Ferula* sp. showed antibiofilm activity against *S. aureus* at increasing concentrations. In addition, the methanol extract showed antibiofilm activity against *C. albicans* at increasing concentrations. Antibiofilm activity graphs are given in Figure 2. While 8 µg/mL ml extract application inhibited biofilm formation in *C. albicans*, 32 µg/mL ml extract application reduced biofilm formation in *S. aureus* by half. Consequently, *Ferula* sp. extract inhibits *C. albicans* biofilm formation at a value four times lower than the MIC value. This shows that it contains molecules with strong antibiofilm effect.

Table 1. Zone diameter (mm) and MIC values (µg/mL) for pathogens

Patogens	Zone diameter (mm)	MIC value (µg/mL)
S. aureus (ATCC 25923)	21	16
E. coli (ATCC 25922)	18	32
C. albicans (ATCC 10231)	15	32



Discussion

Infectious diseases are a significant problem all over the world. In particular, biofilm-related infections cause serious problems and great economic loss. The effect of antibiotics on biofilm-induced infections are

limited (18). Therefore, there is a need for more reliable therapeutic approaches. Ferula sp. extracts have great potential as antimicrobial and antioxidant compounds (8,9). Therefore, it is an essential plant that can be used in therapeutic agents. The aim of this study was to determine whether or not the Ferula species obtained from Erzurum possessed antimicrobial and antibiofilm activity.

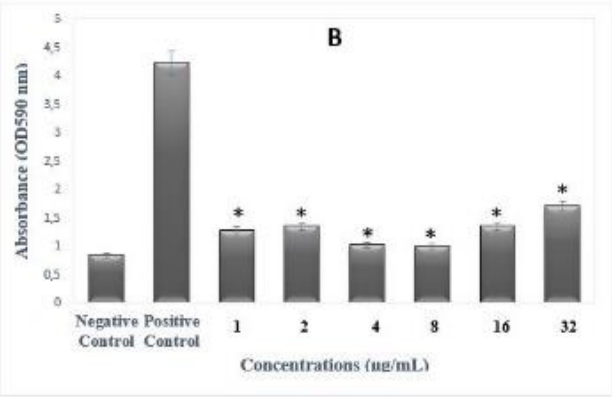


Figure 2. Antibiofilm activity of *Ferula* sp. extracts A. It showed antibiofilm activity at increasing concentrations against *S. aureus*. B. Antibiofilm activity at increasing concentrations against *C. albicans*.

First of all, we performed methanol and ethyl acetate extract of Ferula sp. obtained from the Erzurum province in the current study. Then, we examined the antimicrobial and antibiofilm activities of the obtained extract against the test strains. Many researchers have studied the antibacterial, antifungal and antioxidant properties of Ferula sp. These studies were reviewed in Daneshniya et al, 2021 (18).

Baldemir et al, in 2006, investigated the antimicrobial activity of the methanol extract of the Ferula halophila against various pathogens by disc diffusion method. Chloroform extract of F. halophila did not show activity against E. faecalis, E. coli, P. aeruginosa, and C. albicans. However, its methanol extracts showed a weak effect against Bacillus subtilis, Bacillus cereus and S. aureus (19). We obtained similar results in our study. One reason for the lack of antimicrobial activity of Ferula sp. against P. aeruginosa, E. faecalis, and C. tropicalis maybe that Ferula sp. contain volatile compounds with antimicrobial activity (20). Another reason may be the degradation of a molecule effective against these pathogens during extraction.

Alipour et al. (2014) showed that essential oils of Ferula cupularis have high antimicrobial activity against S. aureus, S. epidermidis, B. subtilis, E. coli, K. pneumoniae, and P. aeruginosa. Also, In addition, the most increased activity was obtained from the roots of F. cupularis (21).

Bhatnagar et al. (2015) found that polar extracts showed more antimicrobial activity than nonpolar extracts in their study with Ferula asafoetida (14). Daneshkazemi et al. (2019) showed that F. assa-foetida extracts are antimicrobial against oral pathogens such as Streptococcus mutans and Streptococcus sanguinis (22).

Karakaya et al. in 2019 showed that essential oils from the aerial parts of F. orientalis obtained from Erzurum were effective against S. aureus and C. albicans. Similar results were obtained in our study. However, in our study, unlike in the article, antimicrobial effects were also observed on E. coli. One reason for this is that we may have worked with different subspecies of Ferula (24).

One of the most important causes of antibiotic resistance is biofilm formation. Therefore, molecules that inhibit biofilm formation need to be discovered. Plant extracts are thought to be rich in molecules with antibiofilm activity. To our knowledge, there is only one study of antibiofilm activity against Candida species. Zomorodian et al, (25) showed that essential oils of F. assafoetida have antibacterial and antifungal activity. They also showed antibiofilm activity on C. albicans, C. tropicalis, and C. krusei. They stated that 4 µL/mL ml essential oil completely inhibited biofilm formation (23). Similarly, we observed antibiofilm activity against C. albicans in our study. Our best result was obtained with 8 µg/mL ml extract. This result indicates that the

concentration required for biofilm inhibition is lower than the MIC value. This shows that Ferula sp. are effective candidate molecules for infections caused by C. albicans biofilm.

Furthermore, the antibiofilm activity of Ferula extract against S. aureus was demonstrated for the first time in this study. Ferula extract inhibited half of the biofilm formation at a value of 32 µg/mL ml. Therefore, Ferula sp. are therapeutic candidate molecules in biofilm-induced infections of S. aureus. However, synergism with different antimicrobials or antibiotics needs to be examined.

In this study, antimicrobial and antibiofilm activities of *Ferula* sp. collected from the Erzurum region were investigated. Antimicrobial activity of *Ferula* sp. against *S. aureus*, *E. coli* and *C. albicans* was observed. It was also found to have antibiofilm activity by increasing concentrations against *S. aureus* and *C. albicans*. This is the first study to show the antibiofilm activity of *Ferula* sp. against *S. aureus*. Further characterization of *Ferula* sp. and our understanding of the nature of their antibiofilm properties are needed.

Acknowledgements

This work was supported by the Tubitak 2209-A undergraduate research project conducted by Şimal Topçu.

Declaration of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions: ŞT, EA, ŞÇ, AY and Üİ contributed to the study conception and design. ŞT, EA, ŞÇ and AY contribution to laboratory work. Writing the article (AY and Uİ). All authors read and approved the final manuscript. ŞT:Şimal Topçu, EA: Elif Aslan, ŞÇ: Şeymanur Çobaoğlu, AY: Ayşegül Yazıcı, Üİ: Ümit İncekara.

ORCID

Şimal Topçu 0000-0002-0896-4205
Elif Arslan *0000-0001-7310-241X*
Şeymanur Çobanoğlu 0000-0002-2805-0523
Ayşenur Yazıcı 0000-0002-3369-6791
Ümit İncekara 0000-0002-3283-5841

References

1. Cascioferro S, Carbone D, Parrino B, Pecoraro C, Giovannetti E, Cirrincione G, Diana P. Therapeutic strategies to counteract antibiotic resistance in MRSA biofilm-associated infections. Chem Med Chem 2021; 16;1: 65-80.
2. Iscan G, Demirci F, Kirimer N ,et al .Antimicrobial screening: Mentha piperita essential oil. J Agric Food Chem 2002;50:39433946.
3. Sokovic MD, Vukojevic J, Marin PD, et al. Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. Molecules 2009;1;4;1:238–249.
4. Kanatt S, Chander R, Sharma A. Chilean and mint mixture: a new preservative for meat and meat products. Food Chem 2008;107: 845–852.
5. Tajkarimi MM , Ibrahim SA, O D. Cliver antimicrobial herb and spice compounds in food. Food Cont 2010;21 1199–1218.
6. Yang SA, Jeon SK, Lee EJ, Shim CH, Lee IS. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. J Nat Prod Res 2010;24;2:140–151.
7. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr 2005; 56;4:287–291.
8. Li GZ, Wang J.C, Li XJ, Li Cao An unusual sesquiterpene coumarin from the seeds of Ferula sinkiangensis. J Asian Nat Prod Res 2016;18-9.
9. Zellagui A, Gherraf N, Hegazy MEF, Akkal S, Rhouati S, Dendougui H, et al. Phytochemical investigation and antimicrobial activity of crude extract of the roots of ferula vesceritensis. Chem Nat Compd 2012;48,5.
10. Pavlovic I, Petrovic S, Radenkovic M, Milenkovic M, et al. Composition, antimicrobial, antiradical and spasmolytic activity of Ferula heuffelii Griseb. ex Heuffel (Apiaceae) essential oil. Food Chem 2012; 130, 310–315.
11. Kavoosia G, Rowshan V. Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from Ferula assa-foetida oleo-gum-resin: Effect of collection time. Food Chem 2013;138 2180–2187.
12. Duman H, Sağiroğlu MA .New species of Ferula (Apiaceae) from South Anatolia, Tur- key. Bot. J. Linn. Soc 2005;147, 357-361.
13. Sağiroğlu MA, Duman H. Ferula mervynii (Apiaceae), a distinct new species from north-east Anatolia, Turkey. Bot J Linn Soc 2007;153, 357-362.
14. Richa B , Rani R, Dang AS. Antibacterial activity of Ferula asafoetida: a comparison of red and white type. Journal of Applied Biology and Biotechnology 3.2 2015: 0-2.
15. Rahman MU, Gul S, Odhano EA. Antimicrobial activities of Ferula assafoetida oil against gram positive and gram negative bacteria. Am-Euras J Agric Environ Sci 4.2 2008: 203-6.
16. European committee on antimicrobial susceptibility testing. MIC distributions and epidemiological cut-off value (ecoff) setting, eucast sop 10.1, 2019 Available from: http://www.eucast.org.
17. Üstün A, Yazıcı A, İskender NA, Örtücü S. The evaluation of antimicrobial and antibiofilm activity of bioactive compounds obtained from Aspergillus Sclerotiorum. JIST, 9.3 2019: 1666-1673.
18. Daneshniya M, Maleki MH, Mohammadi MA, Ahangarian K, Kondeskalaei V J, Alavi H. Antioxidant and antimicrobial activity of Ferula Species essential oils and plant extracts and their application as the natural food preservatives. J Asian Nat Prod Res 2021;4: 3:1-23.
19. Baldemir A, Coşkun M, Yıldız S, Antimicrobial activity of Ferula halophila Peflmen. Fabad J Pharm Sci 2006;31:57-61.
20. Mareshah A, Schroeder S, Goelz L, Sulser A, Rose TS, Puchalski K Langland J, et al. Antimicrobial activity of the volatile substances from essential oils. BMC Complement Med Ther 2021; 21:1,1-14.

21. Alipour Z, Taheri P , Samadi N. Chemical composition and antibacterial activity of the essential oils from flower, leaf and stem of Ferula cupularis growing wild in Iran. Pharm Biol 2015; 53:4, 483-487.
22. Daneshkazemi A, Zandi H, Davari A, Vakili M, Emtiazi M, Lotfi R, Masoumi SMR. Antimicrobial Activity of the essential oil obtained from the seed and oleo-gum-resin of Ferula Assa-Foetida against oral pathogens. Front Dent 2019;16:2:113-120.
23. Zomorodian K, Saharkhiz J, Pakshir K, Immeripour Z, Sadatsharifi A. The composition, antibiofilm and antimicrobial activities of essential oil of Ferula assafoetida oleo-gum-resin. Biocatal Agric Biotechnol 2018;14:300-4.
24. Karakaya S, Göger G, Bostanlik FD, Demirci B, Duman H, Kilic CS. Comparison of the essential oils of Ferula orientalis L., Ferulago sandrasica Peşmen and Quézel, and Hippomarathrum microcarpum Petrov and their antimicrobial activity. Turkish J Pharm Sci 2019; 16:1- 69.
25. Sharma, Divakar; Misba, Lama; Khan, Asad U. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob. Resist. Infect. Control 2019, 8.1: 1-10.

Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

The potential role of long non-coding RNAs and micro RNAs in insects: From junk to luxury

Insha Shafi[1], Mudasir Gani[1*], Sadiah Shafi[2], Taskeena Hassan[3], Mohd. Ayoub Mantoo[1], Kamlesh Bali[4], Rakesh Kumar Gupta[4]

[1]*Division of Entomology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura, Sopore – 193 201, Kashmir, J&K, India*
[2]*Division of Genetics & Plant Breeding, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Wadura, Sopore – 193 201, Kashmir, J&K, India*
[3]*Department of Zoology, Aligarh Muslim University, Aligarh – 202 002, Uttar Pradesh, India*
[4]*Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha – 180 009, Jammu, J&K, India*

Cite: *Shafi I, Gani M, Shafi S, Hassan T, Mantoo M.A, Bali K, Gupta R.K, The potential role of long non-coding RNAs and micro RNAs in insects: From junk to luxury. Eurasian Mol Biochem Sci 2022;1(1): 28-37.*

Received: 04 April 2022, Accepted: 13 April 2022 *DOI. 10.54672/ejmbs.2022.6*

Abstract

Noncoding RNAs (ncRNAs) play an important role in almost all biological processes and comprise a layer of internal signals that have a potential control on various levels of gene expression. The research in the field of ncRNAs has progressed a lot owing to the recent developments in sequencing methods and information analysis. A large number of ncRNAs have been identified in insects from the RNA-Seq data or transcriptomes and they have important regulatory functions at the epigenetic, transcriptional, or post-transcriptional levels. The technological innovations have made it possible to discover ncRNAs from both beneficial (honeybees, silkworm, etc.) and harmful insects (Plutella xylostella, Helicoverpa armigera, Bactrocera species, aphids etc.). The characterization and utilization of ncRNAs in the field of insect science have become a worldwide research focus and they are believed to have potential applications in insect pest management and the prevention and management of diseases of beneficial insects.

Keywords: Gene regulation, Insect, Pest, Management, ncRNA, miRNA.

1. Introduction

Ribonucleic acid (RNA) molecules were thought to be nothing more than a messenger between DNA and protein, but that is no longer the case as a messenger between DNA and protein for decades, but now it's well established that they have key role in almost all biological processes. There are broadly two types of RNAs viz. Coding and Non-coding. The coding RNAs generally refer to RNAs which encode proteins and it includes the messenger RNA (mRNA) only. Non-coding RNA molecules (ncRNAs) arise from the transcription of DNA but do not get translated into protein molecules. The amount of noncoding RNA

* *Corresponding Author:* Mudasir Gani,
Division of Entomology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura, Sopore – 193 201, Kashmir, J&K, India
E-mail: mudasir32@gmail.com

varies among species and proportionally increases with increasing complexity of organism. The ncRNA contains information and remains functionally active by regulating other genes and their function is assessed based on their low coding potential [1]. The regulatory role of non-coding RNAs is thought to be responsible for many of the intricate genetic relationships and differences between species. They have a spatio-temporal expression and are mostly unconserved from species to species. The ncRNAs are involved in various biological and pathological processes [2,3] and can have either DNA binding sites, protein binding sites, or both. The ncRNAs are of many types such as ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), endogenous small interfering RNA (siRNA), PIWI-interacting RNA (piRNA), microRNA (miRNA), long non-coding RNA (lncRNA), circular RNA (circRNA), etc. [4].

The advancement in high-throughput sequencing technologies has gained popularity as a way to determine the level of RNA in cells and provides a snapshot of a cell type or tissue at a specific moment of time. The researchers now can comprehend the complexity and diversity of insect transcriptomic data. According to the recent study, around 156 insect genomes from Diptera, Lepidoptera, and Hymenoptera are sequenced and submitted to the genome database of NCBI [5]. However, the Encyclopedia of DNA Elements (ENCODE) Program revealed that the large chunk of the insect genome generates a plethora of non-protein-coding RNAs, termed as noncoding RNA (ncRNA) [6] (Figure 1). Recent years have seen a paradigm shift as an increasing number of non-coding RNAs has been identified and characterized. The discovery of RNA interference (RNAi) has resulted in a surge of knowledge on the identification of ncRNAs and also how these ncRNAs interact with one another. Non-coding RNAs (ncRNAs) include RNAs that are transcribed from DNA but not translated into proteins. ncRNAs, earlier misidentified as “background noise” or “evolutionary junk”, are emerging as key elements that participate in various biological processes. ncRNAs have gotten a lot of interest recently because of their roles at the epigenetic, transcriptional, and post-transcriptional levels in insects. The recent explosion in science suggests that the repertoire of ncRNAs is still poorly characterized in insects. Here, we provide an overview of two main classes of insect ncRNAs viz., microRNAs and long ncRNAs, and for each class; we describe their biogenesis mechanisms and highlight their certain functions in different insect groups to provide their comprehensive understanding.

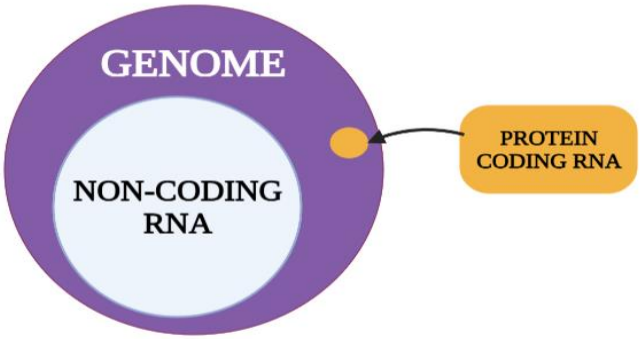


Figure 1. Non-coding RNAs comprise a larger portion of the insect genome.

2. Diversity of insect ncRNAs

The presence of ncRNAs has been witnessed in a broad range of insect species. According to a relatively broad size threshold, ncRNAs have been classified into into two subclasses viz. small ncRNAs and long ncRNAs (Figure 2a). ncRNAs typically less than 200 nucleotides in length are called small or short ncRNAs viz., miRNAs, siRNAs, etc. whereas the which are more than 200 nt in length are called long ncRNAs (lncRNAs). Based on functionality, ncRNAs include regulatory ncRNAs and housekeeping ncRNAs. Ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) fall in the category of housekeeping ncRNAs and regulatory ncRNAs include long non-coding RNAs (lncRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and small nuclear RNAs

(snRNAs) (Figure 2b) [7]. Nonetheless, due to the cross-over of properties, categorization of ncRNAs remains difficult.

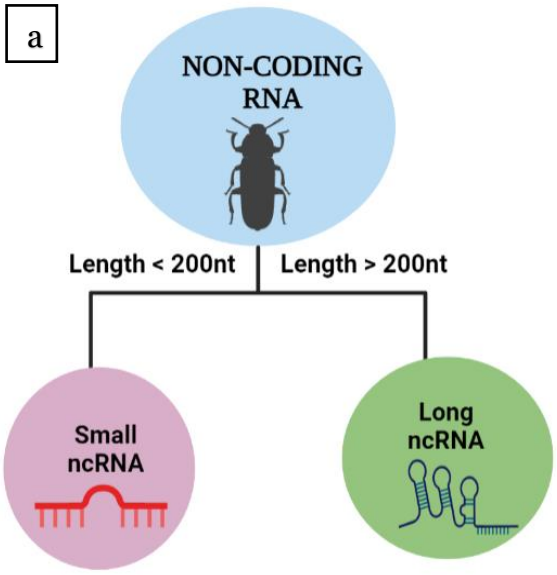


Figure 2a: Classification of ncRNAs based on the size of the transcript

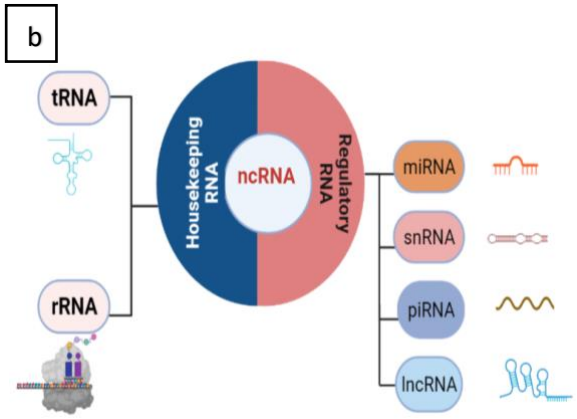


Figure 2b. Classification of ncRNAs based on functionality

3. Identification of ncRNAs

Because of the tremendous transcription power of mammalian genomes and the diverse processes of ncRNA synthesis, the ncRNA world is still loaded of unanswered questions in which unknown RNA species could play some key roles. Thanks to the advancements in technology, more unique functional ncRNAs are being found.

Next-generation sequencing methods, particularly RNA-seq, enable the identification of novel and unusual transcripts such as long noncoding RNAs (lncRNAs) by providing genome-wide expression profiling. Many RNA-seq studies have now been conducted to characterize lncRNAs and their potential role in cell development and differentiation in various animals, cell types, and tissues. RNA-seq is a whole-transcriptome sequencing technique that assesses gene expression across a broad range of values. It has been widely used in the model organism and human research in the past, and it overcomes the limitations of microarray technology. A reference library of approximately 8000 humans long intergenic noncoding RNAs using RNA-seq data has been created by Cabili et al., [8] the vast majority of which had never been described before. Long noncoding RNAs can now be annotated and characterized owing to the recent improvements in RNA-seq and computational techniques for reconstructing transcriptomes. RNA-seq has led to the discovery of a large number of lncRNAs [9]. As a result, enormous amounts of RNA-seq data have enabled us to fully identify and quantify ncRNAs (also protein-coding RNAs), as well as describe their functions. High throughput sequencing was used by Liao et al., [10] to identify a total of 62 lncRNA, 332 miRNA, and 366 mRNA profiles between coronary heart disease and healthy control in humans. Using a combination of next-generation sequencing and bioinformatics, Tonge et al., [11] could identify thousands of lncRNAs located throughout the human genome, based solely on their function using the approach of next generation sequencing technologies aided with bioinformatics.

4. Insect ncRNA research

ncRNAs have been extensively researched in mammals, however their roles in insects are still to be clearly understood. The advent of high-throughput technology has facilitated the sequencing of various insects' genomes and transcriptomes, resulting in the finding of several key ncRNAs in insects. Nowadays,

tens of thousands of ncRNAs have been discovered in insects, thanks to the approaches like RNA sequencing technologies, which provide a basis for the structural and functional research of ncRNA in invertebrates such as insects. Insect ncRNA research previously was focussed on model insects, however, now the researchers are focussing on identifying and deciphering the role of various ncRNAs in non-model insects.

5. MicroRNAs (miRNAs)

MicroRNAs (miRNAs) are small, single-stranded RNAs (ss ncRNAs), about 22-24 nucleotides, with a characteristic hairpin structure. Lin-4 was the first miRNA to be discovered 1993 in Caenorhabditis elegans by the Ambros and Ruvkun groups. Lin-4 was initially thought to be a protein-coding gene, but it was later demonstrated to produce small RNAs and to influence developmental timing in C.elegans [12]. Tens of thousands of miRNAs have been discovered since then, and are now stored in the miRBase database (www.mirbase.org) [13]. Since this first discovery of the lin-4 gene, it has become clear that miRNAs have many different functions. miRNAs are the most studied in humans followed by model organisms from other groups. These miRNAs are believed to serve critical roles in development, apoptosis, cell differentiation, reproduction, behavior, and physiology in eukaryotes, including plants and animals [14-16]. Researchers have previously shown that miRNAs have a key role in a wide range of biological processes in insects, including oogenesis, embryogenesis, mounting, metamorphosis, immunity, behavior, and host-pathogen interactions [17-22]. While miRNA activity is conserved across significantly different species, the functions of miRNAs in the model species fruit fly, Drosophila melanogaster have been thoroughly investigated [23-25]. Understanding the role of miRNA in insects other than fruit fly, on the other hand, is an emerging trend in insect research.

Biogenesis of microRNAs in insects: miRNA biogenesis in insects involves numerous processing steps, viz., Transcription of miRNA loci, Pri-miRNA processing by the microprocessor complex, nuclear export of pre-miRNA, Pre-miRNA processing by Dicer and MicroRNA strand selection, and Argonaute loading (Figure 3). Monocistronic, bicistronic, and polycistronic miRNA transcripts can produce mature miRNAs. These transcripts fold into pri-miRNA viz., primary miRNA hair-loop structures, that are processed in the nucleus by an enzyme, RNase III, which liberates the pre-miRNA, precursor miRNA. Once this pre-miRNA is transferred to the cytoplasm and digested by yet another RNaseIII enzyme, the miRNA-miRNA* pair is synthesized. Although the biogenesis of miRNAs has been extensively researched in model insects such as Drosophila; nonetheless, new findings of non-canonical miRNA production and processing in other species is emerging day by day [26,27].

Steps of miRNA biogenesis:

1. Transcription of miRNA genes

The initial step of miRNA biogenesis is the transcription of miRNA genes. The resulting primary transcripts, also known as primiRNAs, are often several kilobases (kb) long and undergo extensive processing during the formation of the functional 21 nt miRNA [23]. Earlier, it was presumed that RNA polymerase III (pol III) assisted in the transcription of most miRNA sites because it is reported to synthesize the majority of smaller non-coding RNAs including such tRNAs and U6 snRNAs [16]. As suggested by miRNA gene structure and explicit experimental data, miRNA loci appear to act as class-II genes, with polymerase II (pol II) being the principal RNA polymerase facilitating miRNA loci transcription in animal species. Hence, RNA polymerase II transcribes the significant proportion of miRNA genes, while RNA polymerase III transcribes a small percentage of miRNAs. RNA

polymerase III, for example, transcribes miRNAs from the human Chromosome 19 group [26].

2. Processing of primary transcripts (primiRNA) by the microprocessor complex

Pri-miRNAs are usually several kilobases long, with local stem-loop structures, and are polyadenylated and capped, although the cap and the poly (A) tail are removed during miRNA processing [28]. These primiRNA undergo an endonucleolytic cleavage at the stem of the hairpin structure, thereby releasing a 60–70 nt hairpin known as the pre-miRNA by the microprocessor complex. This microprocessor complex (about 500KDa) comprises of an assembly of three components viz., i) an enzyme RNase III, ii) Drosha, and iii) Pasha, its double-stranded RNA (dsRNA) binding partner, otherwise known as DGCR8 in case of mammals and nematode, C. elegans [29-33]. The cleavage of pri-miRNA transcript takes place by the activity of Drosha and its partner protein, Pasha/DGCR8. Pasha/DGCR8 targets the precursor pri-miRNA, hooks to the flanking ssRNA and dsRNA stem junctions, and identifies location 11bp into the stem wherein Drosha's activity centre is located to fragment the pri-miRNA [34]. Drosha-Pasha/DGCR8 processing can be bypassed by a non-canonical class of miRNAs termed miRtrons [35]. Drosha-Pasha/DGCR8 processing can be bypassed by a set of non-canonical of miRNAs termed miRtrons (Ruby et al., 2007). These miRtrons have been heavily studied in Drosophila. The pri-miRNA is converted into pre-miRNA (about 70 nt) by Drosha-Pasha processing.

i. Nuclear export of pre-miRNA

Pre-miRNA is crucial for nuclear transport and is transported into the cytoplasm from the nucleus with the aid of Exportin-5 (Exp-5). Exp-5, a Ran guanosine triphosphate (RanGTP)-dependent dsRNA-binding receptor, enables pre-miRNA outflow by targeting the 2 nt miRNA's 3' tail in the nucleus [36,37]. Exp-5 not only works as an element of nuclear export for pre-miRNAs, but that also shields pre-miRNAs with nuclease degradation [37].

ii. Cytoplasmic processing of pre-miRNA by Dicer

Pre-miRNAs are processed into mature 22 nt miRNA-miRNA* duplexes by Dicer, RNase III type enzyme in the cytoplasm [38]. The miRNA and miRNA* duplexes are the two strands of the dsRNA product of dicer processing of the stem-loop precursor miRNA. Dicer was first discovered in *Drosophila* as an important enzyme in the RNAi pathway [39]. The *Drosophila* genome encodes two Dicer enzymes, Dcr-1 and Dcr-2, each having a unique role in the miRNA and siRNA pathways respectively.

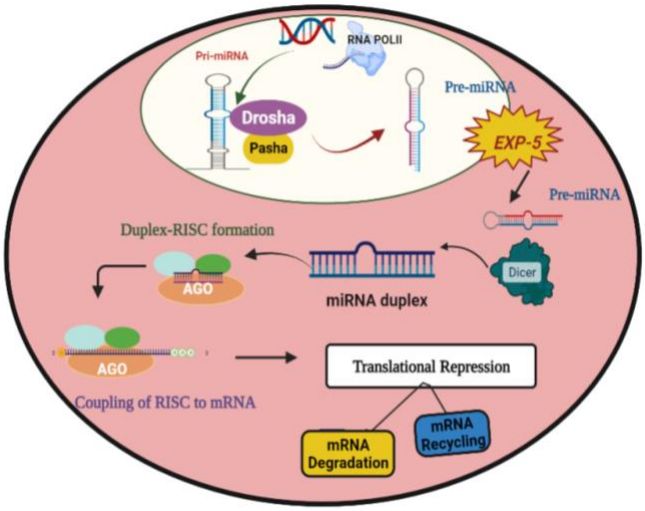


Figure 3. Stepwise Biogenesis of miRNA in insects**, a.** Transcription of miRNA genes into the primary miRNA (pri-miRNA) by RNA polymerase **b.** Processing of pri-miRNA into pre-miRNA by Drosha in association with Pasha **c.** Transport of pre-miRNA into cytoplasm by Exportin 5 **d.** Incorporation of miRNA duplex into the RISC complex followed by translational repression.

iii. Formation of miRNA containing RNA Induced Silencing Complex

After the cleavage of the pre-miRNA to form miRNA-miRNA* duplex by the action of Dcr-1, one of the strands is loaded into the RISC. The Argonaute family of sRNA-guided RNA-binding proteins forms the core part of the RISC. The miRNA-Ago complex is ready to operate on the vast majority of the target sequences of

insect miRNAs; however, certain miRNAs require further processing following Ago loading. Typically, the miRNA-Ago complexes silence the gene expression after transcription by either inhibiting translation or degrading mRNA.

Role of miRNAs in insects:

MiRNAs have an extensive role in various insect biological processes viz., development, immunity, host-pathogen interactions. Some of the miRNA roles in insects have been discussed below:

1.Insect Germ Cell Development

The significance of miRNAs in the developmental processes of insects has received the glaring attention in comparison to other areas of insect biology, owing to their conserved functions in the development of animals. The model insect *Drosophila melanogaster*, which has a plethora of genetic tools at its disposal, has been the focus of miRNA study, particularly concerning insect development. Experimental evidence reveals that proteins involved in controlling germ cell development work in tandem with the miRNA pathway to control important signaling pathways that govern the fate of progenitor cells. In *Drosophila*, loss of miR-184 results in failure of oogenesis coupled with defects in early embryogenesis [40]. For proper differentiation of germ stem cells (GSCs) in *Drosophila,* the repression of miR-7 caused by Maelstrom protein is a must and the absence of which inhibits differentiation of GSCs into primary spermatocytes in the testes [41]. miRNAs have a role to play in the panoistic type of oogenesis in *Drosophila,* apart from the meroistic type. Experimental findings in the German cockroach, *B. germanica* revealed that miRNAs play an important role in the regulation of oogenesis in panoistic ovaries. The sterile females were found to be developed due to the depletion of Dicer-1 in B. germanica [42].

2.Muscle development

A microRNA, miR-1, regulates heart function in *Drosophila* and mice by maintaining the transcript levels of the RhoGTPase Cdc42 gene, that in turn is essential for cardiac output and the maintenance of myofibrillar architecture in the heart [43]. Besides this, miR-1 is also responsible for keeping the muscular integrity in insects [23]. Because of the profound evolutionary conservation of miRNAs, Drosophila has been used as a model to better comprehend the signaling pathways associated with muscle development, the heart muscles in particular, in order to efficiently manage relevant disorders or ailments.

3.Apoptosis

Research shows that some of the miRNAs viz., Bantam, miR-14, miR-2, and miR-13 supposedly are cell death inhibitors in Drosophila [18]. Besides this, members of the miR-2 family, specifically miR-2/6/11/13/308, are vital for the regulation of cell death during the process of embryogenesis in Drosophila [44].

4.Host-pathogen interactions

The role of insect miRNAs in host-pathogen interactions is now well established [45]. Experimental evidence suggests that miRNAs can target host-pathogen interactions either by targeting the pathogen directly or by changing the expression of host genes which are beneficial to the pathogen [46,47]. In the case of Anopheles gambiae, after the attack and invasion by the parasite Plasmodium berghei, expression patterns of four miRNAs produced in the midgut of the mosquito was significantly modified [48].

5. miRNAs in insect pest management

Only a handful amount of studies, though promising, have been reported on the utilization and successful exploitation of miRNAs for the insect pest management. The trans-kingdom RNA interference (RNAi) approach utilizing miRNAs have been utilized for pest control. This method entails miRNAs being

delivered through kingdoms by food to receptive species, wherein they subsequently execute their biological function. *Escherichia coli has been engineered to express precursors for artificial miRNAs (amiRNAs) that target insects for the purpose of pest control using TK-RNAi/bacterial-mediated miRNA expression. An amiRNA is an altered endogenous miRNA progenitor with sequences tailored to inhibit any predetermined target gene in place of the miRNA: miRNA duplex. Larvae of H. armigera fed with E. coli-expressing a precursor for an amiRNA that targets Ecdysone Receptor EcR, showed considerable mortality, decrease in the rate of oogenesis, and the developmental abnormalities. During feeding, H. armigera is thought to have ingested the E. coli-expressed precursor backbone, which was then changed into the mature amiRNA that targeted EcR and resulted in the visible effects. This strategy is less expensive in comparison to the synthesised miRNA mimics for insect pest control* [49].

6. Long non-coding RNAs (lncRNAs)

NcRNAs of size greater than 200 nt belong to the category of lncRNAs and are believed to be present in all the eukaryotic organisms including insects. LncRNAs are categorized in four groups based on the genomic location from which they are transcribed: a) Sense lncRNAs; b) antisense lncRNAs; c) Intergenic lncRNAs and d) Intronic (bidirectional) lncRNAs [50] (Figure 4). LncRNAs transcribed from the sense strand of protein-coding genes are termed sense lncRNAs. On the contrary, antisense lncRNAs are transcribed from the antisense strand of protein-coding genes. Intergenic lncRNAs are transcribed from Intergenic locations from both the strands while Intronic lncRNAs are transcribed entirely from introns of protein-coding genes. As compared to other classes of ncRNAs in insects, lncRNAs have been extensively studied.

Biogenesis of LncRNAs:

The biogenesis of most of the lncRNAs is similar to that of mRNAs. It is believed that in eukaryotes, the majority of the lncRNAs get transcribed by RNA polymerase II. However, it is found that some of the novel lncRNAs get also transcribed by RNA polymerase III. Most of the lncRNAs are capped, polyadenylated, and spliced by the canonical mode. They can also be metabolized in non-canonical ways, perhaps by cleaving them with ribonuclease P (RNase P) to yield mature 3′ ends, capping them with snoRNA-protein (snoRNP) complexes at their ends, and forming of the circular structures [51].

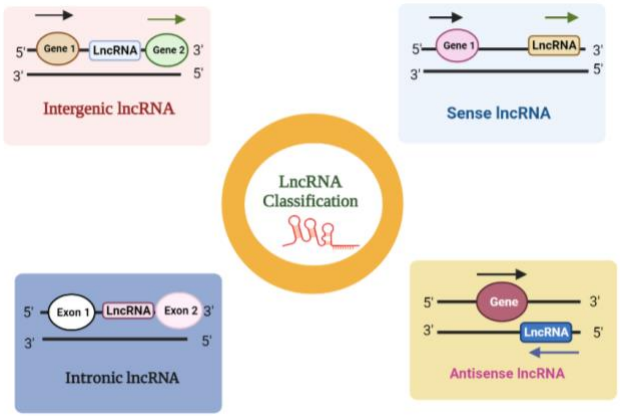


Figure 4. Classification of lncRNAs based on genomic locations.

Mode of action of LncRNA: Three major paradigms for characterizing lncRNA function have emerged: guides, dynamic scaffolds, and molecular decoys.

LncRNAs as Guides: LncRNA as guides are necessary for the appropriate localization and organization of the factors at specific genomic loci for genome regulation. These transcripts connect to regulatory or enzymatically functional proteins, including such transcriptional regulators and chromatin modifiers, to channel them to designated locations in the genome at either cis or trans sites from their transcription locus [52].

LncRNAs as Scaffolds: LncRNAs as "molecular scaffolds", play an important structural role in assembling multi-protein complexes such as short-

lived ribonucleoprotein (RNP) complexes. LncRNAs, which operate as dynamic molecular scaffolds, serve a structural role in the transitory assembly of numerous enzymatic complexes as well as other regulatory co-factors. RNP complexes can repress or enhance transcription depending on the presence and type of the RNAs and proteins involved once they've been fully assembled [53].

LncRNAs as Decoys: The primary purpose of decoy lncRNAs is to act as a molecular sink, limiting the availability of certain regulatory components. RNA-binding proteins, transcription factors, mirnas, catalytic proteins, and components of larger modifying complexes are sequestered by this RNA class, thus modulating the gene expression [54].

Role of lncRNAs in insects: Due to the development of a computational pipeline to identify lncRNAs from RNA-Seq data, a great number of lncRNAs have been identified in insects in these recent years. The role of lncRNAs in the development of insects, insecticide resistance, and anti-viral defense has been studied in various insect pests [55].

i. Dosage compensation and genomic imprinting

i) Dosage compensation and genomic imprinting

Dosage compensation is a technique that organisms use to alter the dose and so balances the X-chromosome (or Z-chromosome) genes expression levels in both males and females of a species. In animals, the dosage compensation acts by epigenetically silencing one of it's x - chromosome in females through the Xist lncRNA in cis form. In Drosophila, the male-specific dosage compensation complex (DCC) activates the majority of genes on the single X chromosome. It is involved in the global acetylation of histone H4 at lysine 16 (H4K16ac), ultimately resulting in a two-fold increase in their expression in order to match the expression from the two X chromosomes in females [56].

ii. Waggle dance in *Apis mellifera*

Based on RNA sequencing of four sets of the honey bee brains, it was discovered that about 2877 lncRNAs and 9647 mRNAs were identified from honey bee brains. The comparison of waggling dancers and non-dancing bees revealed nine differently expressed lncRNAs. A comparison study revealed that two lncRNAs (MSTRG.6803.3 and XR 003305156.1) that were likely engaged in the honey bee waggle dance controlled ten genes via the *cis*-regulatory mechanism [57].

iii. Other functions

LncRNAs are found to be involved in the transition of the larvae during metamorphosis in Drosophila [54]. In Apis mellifera, an abundance of four lncRNA in the brain such as; Nb-1, Ks-1, AncR-1, and kakusei, play a key role in regulating their foraging behavior [58]. Insect lncRNAs have also been implicated in fecundity and pesticide resistance in *N. lugens* [59,60]. Some lncRNAs were shown to be substantially expressed in insecticide-resistant strains [61,62] and some were attributed to chlorantraniliprole resistance in the diamondback moth [61,62].

Conclusion

The ncRNAs are functional and important regulatory molecules in almost all biological processes. The recent advances in the high-throughput sequencing technology has helped the researchers to discover the novel types of ncRNAs and to study their functions. A number of ncRNAs have been reported from insects, but their utilization towards the development of eco-friendly and effective insect pest management practices are lacking. Many studies have also been conducted on the ncRNAs involved in the diseases of beneficial insects such as honeybees and silkworm. Therefore, the research on the structure, function and diversity of ncRNAs in insects deserves special attention to exploit

their role in insect pest management and also in the prevention and management of diseases of beneficial insects.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: IS, MG, SS, TH, MAM, KB and RKG contributed to the study conception and design. IS, MG, SS, TH, MAM, KB contribution to literature work. Writing the article (IS, MG, SS, TH, MAM, KB and RKG). All authors read and approved the final manuscript. IS: Insha Shafi, MG: Mudasir Gani, SS: Sadiah Shafi, TH: Taskeena Hassan, MAM: Mohd. Ayoub Mantoo, KB: Kamlesh Bali, and RKG: Rakesh Kumar Gupta.

ORCID

Mudasir Gani 0000-0003-4410-9924
Taskeena Hassan 0000-0001-5076-4939
Kamlesh Bali 0000-0003-3386-1387
Rakesh Kumar Gupta 0000-0003-3204-7463

References

1- Gardini A, Shiekhattar R. The many faces of long noncoding RNAs. FEBS J 2015; 282:1647-57.
2-Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell 2013;152:1298–1307.
3- Rinn JL, Chang HY. Genome Regulation by Long Noncoding RNAs. Annu Rev Biochem 2012;81:145-166.
4- Lauressergues D, Couzigou JM, Clemente HS, Martinez Y, Dunand C, Bécard G, Combier JP. Primary transcripts of microRNAs encode regulatory peptides. Nature. 2015;2520(7545):90-3.
5- Coordinators NR. Database resources of the national center for biotechnology information. Nucleic acids research 2015;43(Database issue):D6.
6- Hemberger M, Redies C, Krause R, Oswald J, Walter J, Fundele RH. H19 and Igf2 are expressed and differentially imprinted in neuroectoderm-derived cells in the mouse brain. Dev Gene Evol 1998;208:393-402.
7- Dozmorov MG, Giles CB, Koelsch KA, Wren JD. Systematic classification of non-coding RNAs by epigenomic similarity. In BMC bioinformatics 2013; 14:1-12
8- Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & development 2011;25(18):1915-1927.
9- Li X, Xu J, Xiao Y, Ning S, Zhang Y. (Eds.). Non-coding RNAs in Complex Diseases: A Bioinformatics Perspective 2018, (Vol. 1094). Springer.
10- Liao J, Wang J, Liu Y, Li J, Duan L. Transcriptome sequencing of lncRNA, miRNA, mRNA and interaction network constructing in coronary heart disease. BMC medical genomics, 2019;12(1):1-12.
11- Tonge DP, Darling D, Farzaneh F, Williams GT. Whole-genome-scale identification of novel non-protein-coding RNAs controlling cell proliferation and survival through a functional forward genetics strategy. Scientific reports 2022;12(1):1-13.
12- Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75(5):843-854.
13- Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing date. Nucleic Acids Res 2011;39(S1):D152-D157.
14- Ambros V. The functions of animal microRNAs. Nature 2004;431(7006):350-355.
15- Lucas K, Raikhel AS. Insect microRNAs: biogenesis, expression profiling and biological functions. Insect biochemistry and molecular biology 2013;43(1):24-38.
16- Moran Y, Agron M, Praher D, Technau U. The evolutionary origin of plant and animal micro-RNAs. Nat Ecol Evol 2017;1: 0027.
17- Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, Snyder B., et al. The small RNA profile during Drosophila melanogaster development. Developmental cell 2003;5(2):337-350.
18- Asgari S. MicroRNA functions in insects. Insect biochemistry and molecular biology 2013;43(4):388-397.
19- Chawla G, Sokol NS. Hormonal activation of let-7-C microRNAs via EcR is required for adult Drosophila melanogaster morphology and function. Development 2012;139(10):1788-1797.
20- Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. Annual review of microbiology, 2010;64:123-141.
21- Lu LF, Liston A. MicroRNA in the immune system, microRNA as an immune system. Immunology 2009;127(3):291-298.
22- Wei Y, Chen S, Yang P, Ma Z, Kang L. Characterization and comparative profiling of the small RNA transcriptomes in two phases of locust. Genome biology 2009;10(1):1-18.
23- Chawla G, Sokol NS. MicroRNAs in Drosophila development. International review of cell and molecular biology 2011;286:1-65.
24- Luhur A, Chawla G, Sokol NS. MicroRNAs as components of systemic signaling pathways in Drosophila melanogaster. Current topics in developmental biology 2013;105:97-123.
26- Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nature structural & molecular biology 2006;13(12):1097-1101.
27- Waldron JA, Newbury SF. The roles of miRNAs in wing imaginal disc development in Drosophila. Biochemical Society Transactions 2012;40(4):891-895.
28- Belles X, Cristino AS, Tanaka ED, Rubio M, Piulachs MD. Insect MicroRNAs: from molecular mechanisms to biological roles. In Insect molecular biology and biochemistry 2012, pp. 30-56. Academic Press.
29- Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon, GJ. Processing of primary microRNAs by the Microprocessor complex. Nature 2004;432(7014):231-235.
30- Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N., Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004;432(7014):235-240.
31- Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Genes & development 2004;18(24):3016-3027.
32- Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for miRNA biogenesis. Current biology 2004;14(23):2162-2167.
33- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003;425(6956):415-419.
34- Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006;125(5):887-901.
35- Ruby JG, Stark A, Johnston WK, Kellis M, Bartel DP, Lai EC. Evolution, biogenesis, expression, and target predictions of a substantially expanded set of Drosophila microRNAs. Genome research, 2007;17(12):1850-1864.
36- Bohnsack MT, Czaplinski K, Görlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004;10(2):185-191.
37- Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science 2004;303(5654):95-98.
38- Hutvagner G, McLachlan J, Pasquinelli AE, Bálint É, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001;293(5531):834-838.
39-Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001;409(6818):363-366.

40- Iovino N, Pane A, Gaul U. miR-184 has multiple roles in Drosophila female germline development. Developmental cell 2009;17(1):123-133.
41- Pek JW, Lim AK, Kai T. Drosophila maelstrom ensures proper germline stem cell lineage differentiation by repressing microRNA-7. Developmental cell 2009;17(3):417-424.
42- Tanaka ED, Piulachs MD. Dicer-1 is a key enzyme in the regulation of oogenesis in panoistic ovaries. Biol Cell 2012;104(8):452-461.
43- Qian L, Wythe JD, Liu J, Cartry J, Vogler G, Mohapatra B et al. Tinman/Nkx2-5 acts via miR-1 and upstream of Cdc42 to regulate heart function across species. J Cell Biol 2011;193(7):1181-1196.
44- Leaman D, Chen PY, Fak J, Yalcin A, Pearce M, Unnerstall U, et al. Antisense-mediated depletion reveals essential and specific functions of microRNAs in Drosophila development. Cell 2005;121(7):1097-1108.
45- Asgari S. Role of microRNAs in insect host–microorganism interactions. Front Physio 2011;2:48.
46- Pedersen IM, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature 2007;449(7164):919-922.
47- Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. Cell 2009;136(1):26-36.
48- Winter F, Edaye S, Hüttenhofer A, Brunel C. Anopheles gambiae miRNAs as actors of defence reaction against Plasmodium invasion. Nucleic acids research 2007;35(20):6953-6962.
49- Yogindran S, Rajam MV. Artificial miRNA-mediated silencing of ecdysone receptor (EcR) affeTablects larval development and oogenesis in Helicoverpa armigera. Insect biochemistry and molecular biology 2016;77:21-30.
50- Dai X, Kaushik AC and Zhang J. The Emerging Role of Major Regulatory RNAs in Cancer Control. Front Oncol 2019;9:920.
51- Liu Y, Ding W, Yu W, Zhang Y, Ao X, Wang J. Long non-coding RNAs: Biogenesis, functions, and clinical significance in gastric cancer. Molecular Therapy-Oncolytics 2021;23:458-476.
52- Balas MM, Johnson AM. Exploring the mechanisms behind long noncoding RNAs and cancer. Non-coding RNA research 2018;3(3):108-117.
53- Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature, 2012;482(7385):339-346.
54- Chen B, Zhang Y, Zhang X, Jia S, Chen S, Kang L. Genome-wide identification and developmental expression profiling of long noncoding RNAs during Drosophila metamorphosis. Scientific reports 2016;6(1):1-8.
55- Wang Y, Xu T, He W, Shen X, Zhao Q, Bai J, et al. Genome-wide identification and characterization of putative lncRNAs in the diamondback moth, Plutella xylostella (L.). Genomics 2007;110(1):35-42.
56- Conrad T, Akhtar A. Dosage compensation in Drosophila melanogaster: epigenetic fine-tuning of chromosome-wide transcription. Nature Reviews Genetics 2012;13(2):123-134.
57- Feng W, Huang J, Zhang Z, Nie H, Lin Y, Li Z, et al. Understanding of Waggle Dance in the Honey Bee (Apis mellifera) from the Perspective of Long Non-Coding RNA. Insects 2022;13(2):111.
58- Sawata M, Yoshino D, Takeuchi H, Kamikouchi A, Ohashi K, Kubo T. Identification and punctate nuclear localization of a novel noncoding RNA, Ks-1, from the honeybee brain. RNA 2002;8(6):772-785.
59- Xiao H, Yuan Z, Guo D, Hou B, Yin C, Zhang W, et al. Genome-wide identification of long noncoding RNA genes and their potential association with fecundity and virulence in rice brown planthopper, Nilaparvata lugens. BMC genomics 2015;16(1):1-16.
60- Zhu KA, Liu MH, Fu Z, Zhou Z, Kong Y, Liang HW, et al. Plant microRNAs in larval food regulate honeybee caste development. PLoS Genetics 2017;13:e1006946.
61- Etebari K, Furlong MJ, Asgari S. Genome wide discovery of long intergenic non-coding RNAs in Diamondback moth (Plutella xylostella) and their expression in insecticide resistant strains. Scientific reports 2015;5(1):1-14.
62- Liu F, Guo D, Yuan Z, Chen C, Xiao H. Genome-wide identification of long non-coding RNA genes and their association with insecticide resistance and metamorphosis in diamondback moth, Plutella xylostella. Scientific reports 2017;7(1):1-11.

Eurasian Journal of Molecular and Biochemical Sciences
Official Journal of Erzurum Technical University Faculty of Science

AUTHOR INSTRUCTIONS

Introduction

Eurasian Journal of Molecular and Biochemical Sciences (Eurasian Mol Biochem Sci) is national and international, scientific, open access, and periodically published by a double-blind peer-review process. The journal is the official publication of Erzurum Technical University Faculty of science and published biannually in March and September. The publication language of the journal is English.

The Eurasian Mol Biochem Sci aims to publish original research papers, short communications, preliminary scientific reports, letters to the editor, and reviews in all molecular biology and medical fields.

Articles are not limited in length, but the editors recommend that they should be no longer than 15 printed pages with no more than 10 figures and 4 tables in most cases.

Eurasian Journal of Molecular and Biochemical Sciences is an Open Access journal, which means that all content is freely available without charge to the user or his/her institution. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of Open Access.

Editorial and Publication Process

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Peer Review Process

Manuscripts submitted to the Eurasian Journal of Molecular and Biochemical Sciences will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the journal's editorial board members. The Editor in Chief is the final authority in the decision-making process for all submissions.

Ethics in publishing

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors. For manuscripts concerning experimental research on humans, a statement should be included that shows that written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures they may undergo. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. Information on patient consent, the name of the ethics committee, and the ethics committee approval number must also be stated in the Materials and Methods section of the manuscript.

Plagiarism

Eurasian Journal of Molecular and Biochemical Sciences is extremely sensitive about plagiarism. The corresponding author must upload the article's similarity report to the journal's online system. Also, all submissions are screened by a similarity detection software (iThenticate by CrossCheck) at any point during the peer-review and/or production process. Even if you are the author of the phrases or sentences, the text should not have unacceptable similarities with the previously published data.

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act following COPE guidelines.

Language

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical

or spelling errors and conform to correct scientific English may wish to use the English Language Editing services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by email.

Authorship and Author Responsibilities

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Author Contribution

In order to provide appropriate credit to all authors and assign responsibility and accountability for published work, individual contributions should be specified as an Author Contributions statement. The Author Contributions Statement is mandatory in the main text after the discussion. It should represent all the authors and is to be included upon submission. It can be up to several sentences long and should briefly describe the tasks of individual authors. Please list only 2 initials for each author, without full stops, but separated by commas (e.g. JC, JS). Please see an example here:

“AB, CDE, and FG contributed to the conception and design of the study. AB organized the database. CDE performed the statistical analysis. FG wrote the first draft of the manuscript. GH, IJ, AB, and FG wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version”.

Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion, or rearrangement of author names in the authorship list should be made only before the manuscript has been

accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in the author list and (b) written confirmation (email, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of adding or removing authors, this includes confirmation from the author being added or removed.

The Editor will only consider the addition, deletion, or rearrangement of authors in exceptional circumstances after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Declaration of Interest

Eurasian Journal of Molecular and Biochemical Sciences requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. The journal's Editorial Board resolves cases of a potential conflict of interest of the editors, authors, or reviewers within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should contact the editorial office directly regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve claims that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

The statement should be provided as a separate section (titled 'Declaration of interest') at the end of the main text, before the 'References' section.

Disclaimer

Statements or opinions expressed in the manuscripts published in Eurasian Journal of Medicine reflect the views of the author(s) and not the views of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility regarding the published content rests with the authors.

Manuscript Preparation

1- The manuscripts submitted for publication should be prepared in Times New Roman style format, font size 12, A4 paper size, 1.5 line spacing, and 2.5 cm margins of all edges. The legend or caption of all illustrations, such as figures and tables, and their appropriate position should be indicated in the text. Refer to tables and figures in the main text by their numbers. Also, figure legends explanations should be given at the end of the text.

2- Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at **https://dergi.erzurum.edu.tr/ejmbs/about/submissions**. During the submission process, the authors should upload the manuscript's figures to the online manuscript submission system. The **Copyright Transfer Agreement Form** is signed by all the authors and must upload the submission system.

3- The authors should indicate the institute's name approves the necessary ethical commission report and the serial number of the approval in the material and methods section. If necessary, the editorial board may also request the official document of the ethical commission report. If an ethical problem is detected (not reporting project information, lack of ethical committee information, conflict of interest, etc.), the editorial board may reject the manuscript at any stage of the evaluation process.

Manuscripts Types

Original (Full-Article) manuscripts are original and proper scientific papers based on sufficient scientific investigations, observations, and experiments. Manuscripts consist of the title, abstract and keywords, introduction, material and methods, results, discussion, acknowledgment, Declaration of Interest, and references, and it should not exceed 12 pages, including text. The number of references should not exceed 50. The page limit does not include tables and illustrations. The Abstract should contain 250 words. **The template of the Full article main text is here.**

Short communication manuscripts contain recent information and findings in the related topics; however, they are written with insufficient length to be an original full-length article. They should be prepared in the format of the original full-length article, but the Abstract should not exceed 100 words, the reference numbers should not exceed 15, and the text length should be no longer than 6 pages in total.

Preliminary scientific reports are a short description of partially completed original research findings at an interpretable level. These should be prepared in the format of full-length original articles**.**

Letters to the editor are short and picture-documented presentations of subjects with scientific or practical benefits. The length of the text should be no longer than three pages in total. The page limit includes tables and illustrations.

Reviews must include criteria for consideration: a critical overview of the field, with a strong focus on current challenges and the bottlenecks to future major advances. Please make sure to emphasize the molecular mechanistic aspects where possible Otherwise, please feel free to decide the exact scope of your review and the depth of coverage, keeping in mind that the overall message should be accessible to the journal's broad readership. Also, the review must represent a fair and unbiased coverage of the most recent advances and relevant approaches and research groups working in the field and accommodate different points of view. Finally, we would encourage you to include a 'perspectives' paragraph at the end of your review that proposes the direction you personally consider the field should take and the prospects for useful applications you might expect to see in the near future and beyond. The title and summary of this manuscript should be prepared as described for the full-length original articles, and the remaining sections should be followed by abstract, introduction, text (with appropriate titles), conclusion, and references.

Title page

A separate title page should be submitted with all submissions, and this page should include (**Please use Template of Title Page**):

- The full title of the manuscript as well as a short title (running head) of no more than 60 characters,
- Name(s), affiliations, and ORCID IDs of the author(s),
- Grant information and detailed information on the other sources of support,
- Name, address, telephone (including the mobile phone number), and email address of the corresponding author.
- Authors' Contributions (The contributions of all authors should be described on the title page of the manuscript using the CRediT Taxonomy of author roles).

Figures and figure caption

You should not attach figure files in the manuscript or you should upload your figure files to our e-submission system while submitting your paper.

Figures should be submitted in Tiff or JPEJ file format, and they will be automatically attached at the end of the manuscript in PDF. Number figures as Figure 1, Figure 2 ... in order of citation in the figure legend. An individual should not be recognizable in the photographs unless written consent of the subject has been obtained and is provided at the time of submission. Scales should be presented as a bar in the picture or as a magnification remark in the legend.

Except for especially complicated drawings that show large amounts of data, all figures are published at one page or one column width; when the figures are reduced to the size of a single column or of the single page width, the smallest parts of the figure must be legible.

It is recommended to size original figure widths to 4 inches wide. The minimum requirements for digital resolution are:

- 900 DPI/PPI for black and white images, such as line drawings or graphs.
- 300 DPI/PPI for picture-only photographs.
- 600 DPI/PPI for photographs containing pictures and line elements, i.e., text labels, thin lines, arrows.

The abbreviations for a minute, hour, and day are min, hr, and d, respectively, and the abbreviations for a week, month, and year are wk, mo, and yr, respectively. These abbreviations are used in tables, figures, and virgule constructions.

Summary data (e.g., means, odds ratios) should be reported using data markers for point estimates, not bars, and should include error bars indicating measures of uncertainty (e.g., SDs, 95% CIs). Actual values (not log-transformed values) of relative data (for example, odds ratios, hazard ratios) should be plotted on log scales.

The figures should be at least 300 dpi resolution in your Manuscript Word file, and place a caption below each figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used. This is designed to help the reviewers evaluate your work more efficiently.

Tables and table caption

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

Highlights (Optional)

Highlights are optional for this journal. Specifications: include 3 to 5 bullet points (max. 85 characters per bullet point including spaces); only the core results of the paper should be covered. The first bullet point should state the background or context of the question. One to three bullet points should describe the principal results. The last bullet point should conclude with a clear description of the conceptual advance and significance of the work. Highlights should be submitted as a separate file in the Online submission system when uploading files. Highlights will be also in the article PDF file. **An example of Highlights Template is here**.

Abstract

The abstract must be concise (limit of 250 words) and factual. It should convey the concept and the importance of the paper to non-specialist readers. The Abstract should briefly state the background of the question and the principal results and conclude with a clear description of the conceptual advance and significance of the work. Detailed descriptions of the study or the findings should not be included in the abstract. An abstract is required for all papers. An abstract is often presented separately from the article, so it must be able to stand alone. Also, non-standard or uncommon abbreviations should be avoided, but if essential, they must be defined at their first mention in the abstract itself.

Keywords

Authors should supply five keywords after the Abstract. Keywords should not be words from the title.

Abbreviations

Abbreviations should be avoided as much as possible. When they are used, the full expression of the abbreviations in parentheses following the abbreviated word should be given at the first use.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Materials and Methods (primary section heading)

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Subsection headings should be structured hierarchically as follows:

Secondary subsection heading

Tertiary subsection heading

Quaternary subsection heading:

Ethical approval of studies and informed consent

For all manuscripts reporting data from studies involving human participants or animals, formal review and approval, or formal review and waiver, by an appropriate institutional review board or ethics committee is required and should be described in the Methods section.

General text style

Generic and brand name of medicine or chemical.

For medicine, use generic names. If a brand name should be used, insert it in parentheses after the generic name.

Statistical analysis

Standard deviation and the standard error should be described in the format of mean±SD and mean±SE, respectively. *P*-values should be described as $P<0.05$ or $P=0.003$.

Units

Scientific units should be followed by the International System of Units (http://www.bipm.org/en/measurement-units). Unit for volume is 'L', instead of 'l' to avoid confusion; Unit for blood pressure is mmHg; Temperature is expressed in Celsius and concentration in M, mM, and μM.

Results

Results should be clear and concise.

Tables and figures should be indicated in the main text as follows:

(Table 1), (Tables 1 and 2), (Tables 1-3), (Figure 1A and B), (Figure 1A-C), (Figures 1 and 2), (Figures 1-3), (Figures 1A and 3B), (Table 1, Figure 2).

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Acknowledgments

Collate acknowledgments in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title, or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proofreading the article, etc.).

Declaration of Interest

The statement must be provided as a separate section (titled 'Declaration of interest') at the end of the main text, before the 'References' section.

Reference style

References should be listed at the end of the manuscript. They should be listed in the order in which they appear in the text, tables, figure legends, and numbered sequentially. When cited in the text, reference numbers should be given in parenthesis. Only papers that have been published or accepted should be cited in the reference list. If there are six or fewer authors in a reference, then all the names of the authors should be listed. If the number of authors is more than six, list the initial six authors and then abbreviate the rest of the authors with 'et al'., the title of the article, journal title (abbreviated), year of the article, the volume number, and first and last pages should be cited.

Examples:

1- Published articles:

(Author. Title. Journal name Year:Vol;Page.)

1- Kara A, Akman S, Ozkanlar S, Tozoglu U, Kalkan Y, Canakci, CF, et al. Immune modulatory and antioxidant effects of melatonin in experimental periodontitis in rats. Free Radic Biol Med 2013;55:21–26.

2- Kim CS, Choi SH, Chai JK, Cho KS, Moon IS, Wikesjo UM, et al. Periodontal repair in surgically created intrabony defects in dogs: influence of the number of bone walls on healing response. J Periodontol 2004;75:229-35.

Book

(Author. Book title. Edition. Place: Publisher; Year.)

Newman MG, Takei HT, Klokkevold PR, Carranza FA. Carranza's clinical periodontology. 10th ed. St. Louis: Saunder Elsevier; 2006.

Book chapter

(Chapter author. Chapter title. In: editor(s). Book Title. Edition. Place: Publisher; Year. Chapter page.)

1- Hanks SK, Hunter T. The eukaryotic protein kinase superfamily. In the Protein Kinase FactsBook: Protein-Serine Kinases (Hardie, G. & Hanks, S., eds), London, Academic Press, 1995. p.747.

2- Carranza FA, Takei HH. Clinical Diagnosis. In: Newman MG, Takei HT, Klokkevold PR, Carranza FA. Carranza's clinical periodontology. 10th ed. St. Louis: Saunder Elsevier; 2006. p.540-60.

Web site

(Title [Internet]. Place: Publisher; Copyright year [cited Year Month Date]. Available from: URL.)

American Cancer Society. Cancer Reference Information [Internet]. Atlanta (GA): American Cancer Society; c2010 [cited 2010 Jun 20]. Available from: http://www.cancer.org/docroot/CRI/CRI_0.asp.

Other types of references not described below should follow The NLM Style Guide for Authors, Editors, and Publishers (http://www.nlm.nih.gov/citingmedicine.).

Reference management software

These include all products that support Citation Style Language styles, such as EndNote. **The Endnote reference style link is here.**

Publication fee

There are no fees for page charges or color figures.

Offprints

The publisher will send the electronic pdf file to the corresponding author. There are no offprints.

Supplemental data

The acceptance of supplemental material is at the Editor's discretion. Supplemental information must be submitted with the manuscript for review by the editor and referees. Manuscripts must be complete and stand-alone. Supplemental material should complement the printed paper and may include figures and figure legends, tables, supporting data, sequence alignments, primers, derivation of equations, and videos. The availability of supplemental information will be indicated in the printed paper and the supplemental data will be directly linked to the online version of the paper. Reference to the supplemental information may be made at appropriate places in the text.

With the exception of videos, the supplemental information must be submitted electronically in the form of a single PDF file. Very large tabulations of supporting data may be submitted as Microsoft Excel files.

Submission checklist

The following list will be helpful during the final checking of an article before finalizing your submission. Ensure that submission files contain, in the following order:

- Title page
- Highlights (optional)
- Abstract (and Keywords)
- Manuscript main text
- Declaration of authors contribution and funding information in the acknowledgment
- Declaration of interest statement
- References
- Figure captions
- Tables
- Figures
- Copyright Transfer Agreement Form
- Similarity report
-

Submission of Manuscript

Authors are requested to submit their papers electronically by an e-submission system which can be accessed on the official website of Eurasian Mol Biochem Sci (**https://dergi.erzurum.edu.tr/ejmbs/about/submissions**) as well. After creating an account, authors can proceed with the submission process as arranged. Before submission, the instructions described in the Eurasian Mol Biochem Sci manuscript template files should be carefully reviewed in formatting the papers. The title page, manuscript main text (including tables and figures), and copyright form should be included in the submission file package.

Revisions

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format by professional language editors. Once the publication process of a manuscript is completed, it is published online on the journal's

webpage in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.