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In Vitro Anticandidal and Antibiofilm Activities of *Capsella bursa pastoris* Root Against *Candida* Species

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Abstract

This research displays the anticandidal and antibiofilm activities of *Capsella bursa-pastoris* methanol and ethanol extracts against *Candida* species (*C. albicans, C. dupliniensis, C. parapsilosis, C. glabrata* and *C. tropicalis*). Methanol and ethanol extracts of *Capsella bursa-pastoris* were obtained by maceration method. The anticandidal activity of extracts was estimated by agar well diffusion and microdilution assays. The antibiofilm activity of extracts was determined with crystal violet (CV) assay. The root extract *of Capsella bursa pastoris* has an anticandidal activity for all *Candida* species. The flower extract of this plant has the anticandidal activity of *C. albicans*. Microdilution assays showed that plant extract was recorded as >250 mg/L. However, CV assay shows that increasing the concentration of root extracts significantly reduced biofilm formation. The minimum biofilm inhibitor concentration (MBIC) value against *C. tropicalis* was recorded as 64 mg/L. These results suggest that *Capsella bursa-pastoris* root extracts can be used as antibiofilm agents against *Candida* species.

Keywords: Capsella bursa-pastoris root, Anticandidal, Antibiofilm, Extraction, *Candida* species.

Introduction

Medicinal plants have been widely used in the treatment of many diseases throughout human history (1,2) and nowadays, plants are widely used in traditional medicine in Asia and Europe. According to the World Health Organization (WHO), 25% of the pharmaceutical products and drugs used today are obtained from medicinal plants (3). Nowadays, antimicrobial resistance development is dramatically increasing due to the misuse of antimicrobial drugs, biofilm formation, mutation, and gene transfer among bacteria and fungi (4,5). The discovery rate of new antimicrobial drugs is also quite slow (5,6). The most important fungal infection, *Candida* infection, is increasing rapidly all over the world. *Candida albicans* is the most common fungal pathogen. In addition to *C.*

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albicans, other agents such as bacteria, yeast, or fungi cause skin infections in humans and animals. *Candida* species normally live on the skin or inside the body without causing problems. Nevertheless, *Candida* overgrowth and biofilm formation cause infection (6).

Weed compounds can be anticandidal drug candidates due to the bioactive molecules they contain. Indeed, these examples were reviewed by Soliman et al, 2017 (7). Due to the lack of toxic and kinetic information about weed anticandidal products, no drugs are marketed or used in modern therapy yet (7,8). *Capsella bursa-pastoris*, one of the wild herbs, grows in Turkey and is used in traditional medicine (9). The previous report indicated that Capsella bursa-pastoris has antibacterial activity against Gram-positive and Gram-negative bacteria (10).

Notably, its anti-inflammatory, antioxidant and antiulcer activity has been demonstrated (11,12). To our best knowledge, there is no study on the anticandidal activity of *Capsella bursa-pastoris*. Therefore, in this study, we aimed to investigate the potential anticandidal effects of *Capsella bursa-pastoris* extracts against albicans and non-albicans *Candida* species.

Materials and Methods

***Candida* species and Culture Conditions:** In this study, 5 different *Candida* strains (*C. albicans* ATCC 90028, *C. dubliniensis* CBS 7987, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019 and *C. tropicalis* KUEN 1025) were used. All strains were grown on potato dextrose agar (PDA, Oxoid) medium at 30℃ for 48 hours. Liquid cultures for microdilution and antibiofilm activity were performed in potato dextrose broth (PDB, Oxoid) medium at 30 ℃ and 150 rpm. All experiments were performed in at least two replicates.

Preparation of Ethanolic and Methanolic Extracts of *Capsella bursa pastoris:* *Capsella bursa pastoris* was obtained in the local market. About 10 g of the plant (flower, leaf and root, separately) were extracted with 200 mL of methanol and ethanol using the maceration technique (13) for 72 hours at 4℃. After filtration, extracts were evaporated in the device (Rotary Evaporator, Scilogex SCI100). All remaining materials were dissolved in dimethyl sulphoxide (DMSO, Isolab). All extraction was done separately to the flower, leaf and root parts of the plant.

Agar Diffusion Assay: To screen anticandidal compounds of *Capsella bursa pastoris* extract, agar well diffusion assay was performed (14). All *Candida* species, were inoculated in PDB medium at 30℃ and 150 rpm. After incubation final inoculum was adjusted to 0,12-0,15 optical density at 600 nm. PDA plates were lawn cultured and holes were bored with a cork borer (6 mm). Each well was filled with 200 µL of plant extract (1 mg/mL). DMSO was used as a negative control. All plates were incubated at 30℃, statically. After 24 hours of incubation periods, clear zones around the well were measured.

Microdilution Assay: Microdilution test was performed by the extracts with positive results in agar diffusion according to EUCAST protocol with some modification (15). Briefly, overnight yeast culture was prepared in PDB medium at 30 ℃ and 150 rpm. After incubation final inoculum was adjusted to 0,08-0,1 optical density at 600 nm. In a 96-well plate, 100 µL of the extract with increasing concentration (0.5-128 mg/L) was added and 100 µL of yeast culture was added. The final volume was adjusted to 200 µL. PDB medium was used as a negative control. Eventually, the plates were incubated statically at 30°C for 24 hours. At the end of the period, the concentration with no yeast growth was determined as MIC value.

Antibiofilm Activity: For the antibiofilm assay, CV assay was used with some modifications (16). Firstly, yeast culture was started in a similar way to the microdilution test as described above in the 96-well plate. After 48 hours of incubation, all free cell contents were removed and the wells were washed with sterile water. Then, 0,1% crystal violet (CV) dye was added in

each well for 20 min. After that, CV dye was removed. All wells were washed with tap water. Finally, 30% acetic acid was added to each well, and absorbance was measured at 590 nm with a spectrophotometer (Thermo, Multiscan).

Results

In the current study, three different parts (flower, leaf and root) of *Capsella bursa pastoris* were extracted separately. The anticandidal activity of the extracts against *Candida* species was determined by agar well diffusion and microdilution assays. In the agar diffusion test performed for flower extracts, it was observed that a zone was observed in the ethanol flower against *C. albicans* (15 mm). In the leaf extract, no results were obtained. On the other hand, for *Capsella bursa pastoris* root extract, zone formation was observed against all *Candida* species (15-18 mm) Figure 1 shows images of selected Petri dishes. Ultimately, we may say that the root extract of *Capsella bursa pastoris* has anticandidal properties against albicans and non-albicans species.

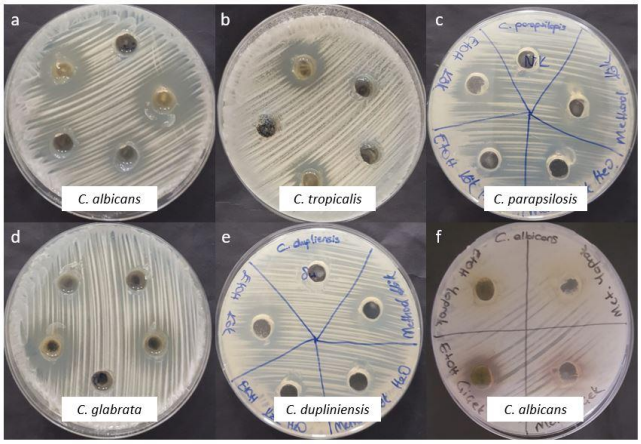

Figure 1: Shows images of selected Petri dishes

The microdilution test was performed to determine the minimum inhibitory concentration (MIC). Growth was found to decrease with increasing concentrations within the concentrations we studied. However, MIC values were not observed and recorded as >250 mg/L. Although the MIC value could not be determined, increasing the concentration of root extracts significantly reduced biofilm formation. Strikingly, the root of *Capsella bursa pastoris* showed antibiofilm activity against *C. tropicalis* and inhibited biofilm formation by 64 µg/mL. The root extract also significantly inhibited *C. albicans* biofilm formation at a value of 256 µg/mL. Therefore, these values were recorded as MBIC values. All in all, Figure 2 shows the CV assay results. Similar results were not obtained in the leaves and flowers of the *Capsella bursa pastoris* weed.

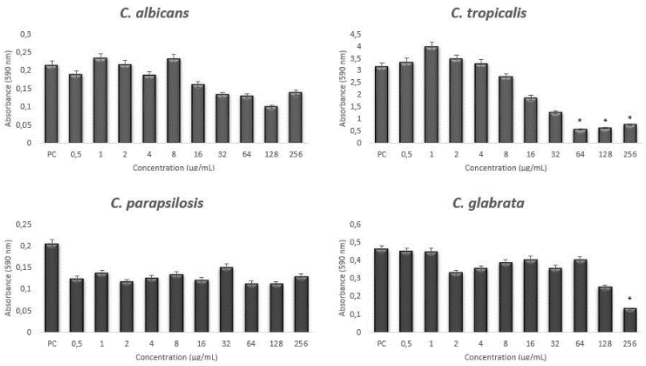

Figure 2: Shows the CV assay results

Discussion

Medicinal plants are used to treat many diseases such as infections and cancer. In particular, herbal treatments for infectious diseases have been applied since ancient times (1-3). In addition, medicinal plants are also the natural material of many drugs.

Capsella bursa-pastoris is a widespread weed belonging to the family Brassicaceae. It has adapted to cold environments and has a short life span. It grows in many regions of Turkey (8). This weed is used in solving reproductive and menstrual problems among people. Taken together, many studies have verified that *Capsella bursa-pastoris* has antioxidant, anti-inflammatory, anticancer and antibacterial effects. *Capsella bursa-pastoris* is used as a wound-healing agent in Korean medicine (11).

Tatçi (1999) reported that extracts of *Capsella bursa-pastoris* prepared with chloroform and hexane showed

antibacterial activity against *Staphylococcus aureus* ATCC 28212 strain (22). In another study, it was reported that water, methanol and ethanol extract of *Capsella bursa-pastoris* had antibacterial activity on *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* (24). On the other hand in a different study reported that ethanolic extract of *Capsella bursa-pastoris* did not show any antibacterial activity against Pseudomonas aeruginosa isolates (25).

Candidal infections have been among the main causes of infections in recent years due to the use of broad-spectrum or combined antibiotics and suppression of the immune system by chemotherapeutic drugs. These infections can be endogenous or exogenous sources of transmission. *Candida* species rank fourth among bloodstream infections occurring in hospitals and intensive care units. They are responsible for 80% of fungal infections occurring in hospitals. The common species encountered in this 80% part is *C. albicans*. Along with the infections that have started to increase in recent years, there is a significant increase in infections caused by non-albicans *Candida* species (17,18).

More than 80% of infections are caused by biofilms. Candida species have the ability to form biofilms, indeed, it is clear that inhibiting *Candida* biofilms is also very important. These biofilms are composed of yeast and hyphae cells and an extracellular matrix. These can colonize mucosal surfaces such as oral and vaginal surfaces (19).

To our knowledge, there is no study on the effect of *Capsella bursa-pastoris* on *Candida* species and Candidal biofilms. We tested this weed's different parts (flower, leaf, root) separately on five different *Candida* species. According to our results, *Capsella bursa-pastoris* showed anticandidal activity against both albicans and non-albicans species. Soleimanpour et al. in their study in 2013, showed the effectiveness of *C. bursa-pastoris* extract against oral pathogens such as *Streptococcus mutans, Actinomyces viscosus, Streptococcus sanguis, and Enterococcus faecalis*. (20). In this study, MIC value was taken for ethanolic extracts. Similarly, in our study, we observed inhibition against *Candida* in ethanolic extracts by agar diffusion test.

In general, the flower and leaf parts of weeds are examined in order to determine bioactive molecules (7). We also included the root part to investigate anticandidal activity in our study. Eventually, we observed anticandidal and antibiofilm activity in root segments in the agar diffusion and CV assay. Taken together, concentration-dependent antibiofilm activity was also observed.

Based on our results, it was verified that *Capsella bursa-pastoris* root extract contains potential compounds against candidal infections. A study supporting this situation was published in 2000 by Park et al (21). Two antimicrobial peptides, shepherin I and shepherin II, were isolated from *Capsella bursa-pastoris* roots. These peptides show antibacterial and antifungal activity against *C. albicans*, *Cryptococcus neoformans*, *Aspergillus flavus* and *Fusarium culmorum*. As this previous study showed, we may have detected anticandidal activity originating from these antimicrobial peptides in the roots of *Capsella bursa-pastoris* collected in Turkey. Another suggestion might be that *Capsella bursa-pastoris* produces chemical compounds with different anticandidal properties. The fact that the leaves and root of *Capsella bursa-pastoris* contain neutral lipids (fatty acids), glucose and phospholipids also support this situation (23). In another study, it was reported that the root parts of this plant are rich in palmitic acid (26). Interestingly, the study published by Prasath et al in 2020 revealed that palmitic acid inhibited the *C. tropicalis* biofilm (27). Collectively, these studies support the anticandidal and antibiofilm activity of *Capsella bursa-pastoris*'s root and inhibition of *C. tropicalis* biofilm.

As a result, this study showed the first time *Capsella*

bursa-pastoris root was shown anticandidal and antibiofilm activity. However, the compounds that cause this activity should be characterized in future studies.

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

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YD, GTB and ŞÇ were made the experiments. AY designed and supervised the experiment. All authors contributed to the writing. This study was supported by Tübitak (2209-A).

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Investigation of Slc30a8 (Rs13266634) Gene Polymorphisms in Type 2 Diabetes Mellitus Patients

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Abstract

Type 2 Diabetes Mellitus (T2DM), a complex metabolic condition, is caused by a number of environmental and genetic factors, as well as their interactions. Many genes associated with T2DM have been discovered through genome-wide association studies. One of these genes is the SLC30A8 gene. T2DM and the SLC30A8 gene are linked to zinc, which is required for insulin secretion and storage. The aim of the research was to look into the relationship between T2DM disease and the rs13266634 polymorphism in the SLC30A8 gene. The research included 80 healthy people and 80 people with type 2 diabetes who were chosen at random from Şanlıurfa. A commercial kit was used to isolate DNA. Following isolation, the SLC30A8 gene region was amplified using PCR and cut with the HpaII enzyme. TT, CT, and CC alleles were discovered after cutting. We used data from 45 diagnosed T2DM cases and 52 confirmed healthy control subjects to detect alleles, and the gel imaging results were reliable and confident. The data analysis was evaluated using the SPSS method. The findings revealed a statistically significant relationship between the patient and control groups and the rs13266634 polymorphism in the SLC30A8 gene. Clarifying the genetic relationship between the rs13266634 polymorphism in the SLC30A8 gene and T2DM disease may aid in identifying genetic predispositions and developing risk profiles.

Keywords: Diabetes mellitus, Type 2 diabetes, polymorphism, SLC30A8 (rs13266634)

Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and impaired carbohydrate, lipid, and protein metabolism, which is caused by a total or partial inadequacy of insulin secretion or action (1). Type 2 Diabetes Mellitus (T2DM), which has a more complicated etiology, is caused by a combination of genetic and environmental factors (2). This condition is caused by changes in insulin secretion and an increase in insulin resistance (3). Although it is estimated that 382 million adults worldwide had T2DM in 2013 (4), when data from research conducted in 130 countries is considered, this figure corresponds to the value projected by the World Health Organization (WHO) for 2030 in its 2004 proclamation (5). The rising prevalence of diabetes, as well as the associated morbidity, mortality, and financial consequences, is a

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major public health issue on a global scale (6).

Through candidate gene approach studies, nearly 70 genes associated with T2DM have been identified in recent years. One of these genes, the 8 (SLC30A8) gene of the soluble carrier family 30 member, has been linked to an increased risk of type 2 diabetes (7). The SLC30A8 gene encodes the zinc carrier protein component-8, which has eight exons and 369 amino acids and regulates -cell zinc homeostasis (8-11). It is required for zinc transfer from the cytoplasm to intracellular insulin-containing vesicles, which is required for insulin maturation, storage, and secretion. The SLC30A8 zinc transporter gene is found in the insulin-secreting granule. The rs13266634 C to T single nucleotide polymorphism in the SLC30A8 gene causes a nonsynonymous mutation at position 325, changing arginine (R) to tryptophan (W) (12). The SLC30A8 gene causes beta cells to malfunction and moves zinc from the cytoplasm to the vesicles that secrete insulin. Zinc is expressed as a result of binding to pancreatic cells. Insulin release is inhibited by the SLC30A8 gene. T2DM is the result of this (11,13). The SLC30A8 gene has also been shown in vitro and in mouse studies to reduce insulin production and cause insulin crystallization (14,15).

The purpose of the study;

i) To better understand the genetic link between the SLC30A8 gene's rs13266634 polymorphism and T2DM disease,

ii) To ensure that genetic predisposition is detected,

iii) To develop risk profiles,

iv) Its goal is to delay the onset of the disease through diagnosis, treatment, and even early intervention.

Materials and Methods

This study was approved by the Harran University Clinical Research Ethics Committee with the ethics committee decision numbered 21.20.11. In our study, 80 T2DM patients and 80 healthy controls were randomly selected from individuals over the age of 18 who applied to Şanlıurfa Harran University Hospital Internal Medicine Outpatient Clinic. Data from 45 diagnosed T2DM cases and 52 confirmed healthy subjects, which gave reliable results in experimental studies, were included in the study. The World Health Organization (WHO) values established in Turkish nationals (126 mg/dl or >7.0 mmol/L) serve as the basis for the inclusion criteria for T2DM patients. Individuals with normal glucose tolerance (FBG>7.0), over the age of 18, and no history of diabetes in the immediate family were eligible to participate as control participants. The study's participants were aged in years and were classified as either male or female.

In our study, blood was collected from both the T2DM patient and control groups and placed in 3 ml EDTA tubes for genotype analysis. Routine biochemical tests were also performed on the blood samples, and the biochemical parameters' values were obtained.

DNA Isolation: The GeneJET Genomic DNA Purification Kit was used to isolate total DNA in this study (Thermo Scientific, USA). During isolation, the kit's protocol steps were followed. To monitor and control the DNA obtained through isolation, DNA samples were collected and stained with 3 l of 2X DNA loading dye. The samples were loaded onto a 1% agarose gel (Sigma Aldrich, Germany) containing 2.5 µl of SYBR Green dye (Applied Biological Materials, Canada), which made the DNA visible under UV light, and then run at 100 V for 30 minutes. The gel imaging system was used to monitor the isolated DNA (Smart View Pro 1100 Imager System, Major Science).

Amplification of the Target DNA Region by PCR: Alharbi et al. (2021) published primer sequences for amplification of the SLC30A08 gene region (F: 5'-GAA GTT GGA GTC AGA GCA GTC-3'; R: 3'-TGG CCT GTC AAA TTT GGG AA-5'). The PCR was carried out in a Thermal Cycler (BIO-RAD T100TM).

Following 3 minutes of initial denaturation at 95°C, 30 seconds of denaturation at 95°C, 30 seconds of bonding

at 50.5°C, and 45 seconds of elongation at 72°C were performed for a total of 35 cycles. Finally, the reaction was stopped after 5 minutes of holding the samples at 72°C. The PCR mixture used in the replication of the target region is; 15.9 µl dH2O, 2.5 µl 1X Taq DNA polymerase buffer (Thermo Scientific, USA), 2 µl MgCl2 (2.5 M), 0.5 µl 10mM dNTP, 1 µl primer (F), 1 µl primer (R), 0.1 µl Taq DNA polymerase (Thermo Scientific, USA) and 2 µl (90 ng) of template DNA, a total of 25 µl.

Treatment of PCR products with the Restriction Enzyme HpaII: For 5 µL of PCR product in the treatment of PCR products with HpaII restriction enzyme; 9 µL of distilled water, 1 µL of 10X Buffer Tango (Thermo Scientific, USA) and 0.5 µL of HpaII (10 U/µl) (Thermo Scientific, USA) enzyme were mixed. The prepared mixture was incubated at 37°C for 3 hours. Inactivation of the HpaII enzyme was carried out at 65°C for 20 minutes. Cut products were loaded onto a 1.5% agarose gel (Sigma Aldrich, Germany) containing which enables the bands of products to be visualized in UV light 3.5 µl of SYBR Green (Applied Biological Materials, Canada) by adding 5 µl of 2X DNA loading dye. 5 µl of GeneRuler 100 base pair Opti-DNA ladder (Applied Biological Materials, Canada) was used as ladder. The gel was run at 90 V for 30 minutes. After running, the bands were visualized by using the gel imaging system (Smart View Pro 1100 Imager System, Major Science) (Figure 1).

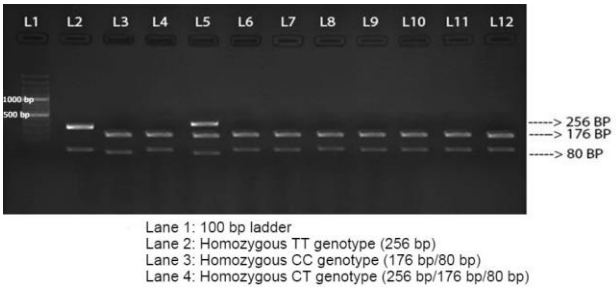

Figure 1. The image of PCR products under UV light as a result of interference with the restriction enzyme HpaII

Sequence Analysis: Following the identification of the individuals' genotypes, randomly chosen samples were sent for sequencing analysis to verify the precision of these genotypes. In Figure 2, the image of the analysis result of the gene sequences obtained from the FinchTV computer program is shown. The peaks in the base sequences obtained with the FinchTV program were carefully examined.

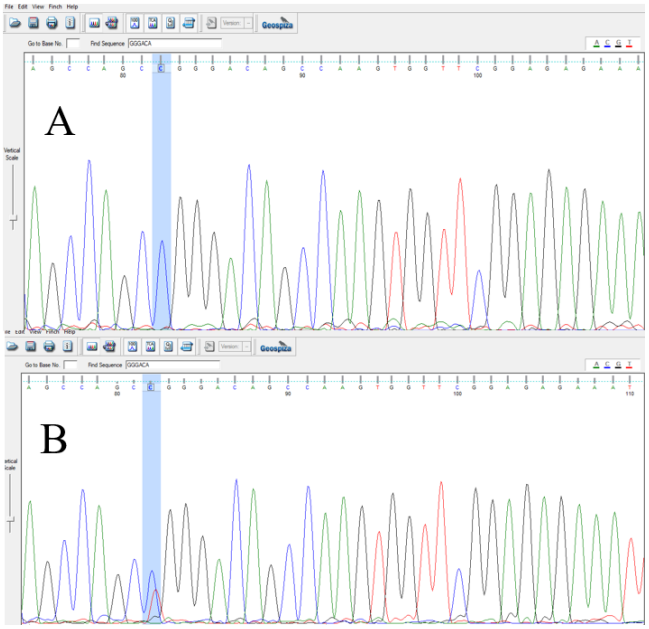

Figure 2. A) Sequence image of CC genotype, B) Sequence image of CT genotype

Statistical Data Analysis: Data analysis was done using SPSS software, version 24.0, which stands for Statistical Packages for Social Sciences. The Mann-Whitney U test and chi square test was employed for the parameters that did not fit the normal distribution criteria, whereas the t test was utilized for the values that satisfied the condition.

Results

Biochemical Results: According to the analysis results of uric acid, ALT, NA, K, WBC, HGB, and PLT parameters, it was determined that there was no significant link between T2DM patients and the control group ($p>0.05$) (Table 1).

Table 1. Comparison of study groups in terms of parameters using t test (n: number of individuals, sd: standard deviation, t: t test, p: p value)

		n	mean	sd	min	max	t	P
Age	Patient	45	57.29	15.43	-	-	**1.131**	**0.261**
	Control	52	53.96	13.56				
Uric acid	Patient	45	4.99	1.98	4	8	**0.021**	**0.983**
	Control	52	4.98	1.92				
ALT	Patient	45	30.42	20.56	5	33	**-0.573**	**0.568**
	Control	52	33.37	28.63				
Na	Patient	45	137.87	3.68	136	145	**-1.127**	**0.263**
	Control	52	138.75	3.99				
K	Patient	45	4.50	0.57	3,5	5,1	**1.869**	**0.065**
	Control	52	4.27	0.61				
WBC	Patient	45	9.16	2.86	4,3	10.3	**0.026**	**0.979**
	Control	52	9.14	3.90				
HGB	Patient	45	12.66	3.12	12	14	**-0.051**	**0.959**
	Control	52	12.69	2.44				
PLT	Patient	45	290.42	87.70	150	450	**0.374**	**0.709**
	Control	52	282.21	122.55				

There is a statistically significant difference between the control group and T2DM patients when the analytical findings of the glucose, urea, and creatinine parameters are examined ($p<0.05$). These levels are lower in the controls than in the patients when the averages of these parameters are taken into account. There was no statistically significant difference between T2DM patients and the control group in terms of GGT and amylase parameters ($p>0.05$) (Table 2).

Table 2. Comparison of study groups in terms of parameters using Mann Whitney U test (n: number of individuals, sd: standard deviation, U: Mann Whitney U test, p: p value, *: p<0.05)

		n	mean	Sd	min	Max	U	p
Glucose	Patient	45	198.47	94.84	82	115	**-7.519**	**0.000***
	Control	52	91.50	6.16				
Urea	Patient	45	47.43	28.41	17	49	**-2.198**	**0.028***
	Control	52	39.91	27.61				
Creatinine	Patient	45	1.24	1.28	0,5	0,9	**-2.532**	**0.011***
	Control	52	0.84	0.57				
GGT	Patient	45	55.44	60.11	40	80	**-1.205**	**0.228**
	Control	52	59.65	101.97				
Amylase	Patient	45	63.46	32.63	40	90	**-0.423**	**0.672**
	Control	52	71.23	74.52				

Genotype Analysis Results: The correlation between genotype and genotype frequencies in T2DM patient and control groups for the SLC30A8 gene's rs13266634 polymorphism is shown in Table 3. The genotypes of 45 people with T2DM and 52 people in the control group were determined at random. The frequency of the CC, CT, and TT genotypes in T2DM patients is 47.2%, 20.0%, and 80.0%, respectively, compared to 52.8%, 80.0%, and 20.0% in controls. The findings of the study revealed that the incidence of TT was higher in the T2DM patient group, while the incidence of CT was higher in the control group. There was no statistically significant relationship between the groups or gender ($p>0.05$).

Table 3. Genotype relationship between T2DM patients and control groups (n: number of individuals, X2: chi-square test, p: p value, *: p<0.05)

		Patient		Control		Chi-square test	
		n	**%**	**N**	**%**	X^2	**p**
Genotype	CC	34	47.2	38	52.8	**0.002**	**0.781**
	CT	3	20.0	12	80.0	**3.793**	**0.050***
	TT	8	80.0	2	20.0	**5.064**	**0.027***

The results of the study show that people with the CC genotype in the T2DM patient and control groups have statistically significant differences in glucose, urea, and creatinine levels ($p<0.05$). Furthermore, the control group's averages of these values were lower than the T2DM patient group's averages (Table 4). Other than glucose, urea, and creatinine, there is no statistically significant difference between the T2DM patient group and the control group among those with the CC genotype ($p>0.05$). The average K and PLT values in the T2DM case group are higher than in the control group. For all other parameters, it is possible to conclude that the T2DM case group had lower averages than the control group (Table 4).

Table 4. Comparison of biochemical parameters of T2DM patient group with CC genotype and control group (Ref: reference, sd: standard deviation, p: p value, a: t test, b: Mann Whitney U test, *: $p<0.05$)

	CC							
	Patient		Control		Ref. Values		Comparison Test	
	mean	**sd**	**mean**	**Sd**	**min**	**max**	**Test Statistics**	**P**
Age	56.65	15.13	52.87	13.36	-	-	**1.125[a]**	**0.264**
Uric acid	4.99	1.91	5.11	2.08	4	8	**-0.252[a]**	**0.801**
ALT	30.76	21.02	36.89	31.49	5	33	**-0959[a]**	**0.341**
Na	137.76	3.79	138.66	4.53	136	145	**-0.902[a]**	**0.37**
K	4.49	0.56	4.33	0.62	3,5	5,1	**1.119[a]**	**0.267**
WBC	9.28	2.93	9.56	4.22	4,3	10,3	**-0.317[a]**	**0.752**
HGB	12.37	3.22	12.65	2.47	12	14	**-0.422[a]**	**0.675**
PLT	276.79	82.69	274.82	125.23	150	450	**0.078[a]**	**0.938**
Glucose	204.41	102.51	91.05	6.29	82	115	**-6.263[b]**	**0.000***
Urea	48.65	28.7	41.88	31.12	17	49	**-2.147[b]**	**0.032***
Creatinine	1.36	1.43	0.81	0.63	0,5	0,9	**-3.204[b]**	**0.001***
GGT	49.88	50.4	69.97	117.26	40	80	**-0.908[b]**	**0.364**
Amylase	58.2	24.15	70.37	81.77	40	90	**-0.034[b]**	**0.973**

There is a statistically significant difference in glucose levels between people with the CT genotype in the T2DM patient and control groups ($p<0.05$). Furthermore, the mean glucose value for the T2DM patient group is higher than the mean for the control group (Table 5).

Table 5. Comparison of biochemical parameters of T2DM case group with CT genotype and control group (Ref: reference, sd: standard deviation, p: p value, a: t test, b: Mann Whitney U test, *: $p<0.05$)

	CT							
	Patient		Control		Ref. Values		Comparison Test	
	mean	**sd**	**mean**	**Sd**	**min**	**max**	**Test Statistics**	**P**
Age	48.67	17.1	55.75	15.02	-	-	**-0.715[a]**	**0.487**
Uric acid	5.67	1.91	4.57	1.5	4	8	**1.088[a]**	**0.296**
ALT	29.33	18.01	23	17.34	5	33	**0.563[a]**	**0.583**
Na	140	2	138.83	2.17	136	145	**0.844[a]**	**0.414**
K	4.67	0.23	4.18	0.6	3,5	5,1	**1.339[a]**	**0.203**
WBC	10.51	4.51	8.1	2.84	4,3	10,3	**1.183[a]**	**0.258**
HGB	14.37	2.31	12.59	2.54	12	14	**1.099[a]**	**0.292**
PLT	297	45.04	301.08	126.74	150	450	**-0.054[a]**	**0.958**
Glucose	125.67	16.01	92.08	5.2	82	115	**-2.603[b]**	**0.009***
Urea	33.53	9.88	33.17	12.63	17	49	**-0.436[b]**	**0.663**
Creatinine	0.73	0.12	0.91	0.38	0,5	0,9	**-0.443[b]**	**0.658**
GGT	19	9.85	33.33	23.68	40	80	**-1.156[b]**	**0.248**
Amylase	77.33	18.93	67.67	47.71	40	90	**-0.866[b]**	**0.386**

Other than glucose, there was no statistically significant difference between the T2DM patient group and the control group among those with the CT genotype (p>0.05). Furthermore, while the average PLT, creatinine, and GGT readings in the T2DM patient group were lower than those in the control group, the averages of the other parameters were found to be higher (Table 5).

Among people with the TT genotype, there was no statistically significant difference in parameters between the T2DM patient group and the control group (p>0.05). T2DM patients with the TT genotype have higher ALT, K, WBC, PLT, glucose, urea, and GGT levels than the control group. Aside from these measures, the averages of uric acid, Na, HGB, creatinine, and amylase levels in T2DM patients are lower than in the control group (Table 6).

Table 6: Comparison of biochemical parameters of T2DM case group with TT genotype and control group (Ref: reference, sd: standard deviation, p: p value, a: t test, b: Mann Whitney U test, *: p<0.05)

	TT							
	Patient		Control		Ref. Values		Comparison Test	
	mean	**sd**	**mean**	**sd**	**min**	**Max**	**Test Statistics**	**P**
Age	63.25	16.07	64	1.41	-	-	**-0.130[a]**	**0.900**
Uric acid	4.74	2.45	5.05	1.06	4	8	**-0.170[a]**	**0.869**
ALT	29.38	21.88	28.5	2.12	5	33	**0.054[a]**	**0.958**
Na	137.5	3.74	140	0	136	145	**-0.904[a]**	**0.393**
K	4.48	0.75	3.65	0.07	3,5	5,1	**1.483[a]**	**0.176**
WBC	8.14	1.82	7.53	0.43	4,3	10,3	**0.457[a]**	**0.660**
HGB	13.26	2.99	14	2.26	12	14	**-0.321[a]**	**0.756**
PLT	345.93	105.31	309.5	33.23	150	450	**0.464[a]**	**0.655**
Glucose	200.5	66.98	96.5	10.61	82	115	**-1.828[b]**	**0.068**
Urea	47.45	32.76	42.8	24.21	17	49	**0.000[b]**	**1.000**
Creatinine	0.95	0.57	1	0.28	0,5	0,9	**-0.793[b]**	**0.428**
GGT	92.75	91.65	21.5	12.02	40	80	**-1.306[b]**	**0.192**
Amylase	80.63	57.34	109	87.68	40	90	**-0.522[b]**	**0.602**

Discussion

The polymorphism rs13266634 was found in this study's analysis of SLC30A08 gene sequences from both the T2DM patient and control groups. When the genotypes of the patient and control groups were compared, the genotypes of the two groups showed a statistically significant correlation with the groups (p<0.05). T2DM patients had a higher prevalence of the TT genotype (80.0%) than the CT genotype (80.0%) in the control group (80.0%).

In our study, we looked at biochemical variables like glucose, urea, and creatinine, as well as the relationship between genotype and study groups. When the two groups were compared, there was a statistically significant difference (p<0.05) between the control group and the T2DM patient group. When these numbers were compared as T2DM individuals versus controls, it was discovered that glucose (198.47/91.50), urea (47.43/39.91), and creatinine (1.24/0.81) had average values and were lower in the control group. The fact that the analyses performed in our study revealed no significant association between genotype and gender adds to the credibility of our study.

Wu et al. (2008) investigated the relationship between the SLC30A8 gene and T2DM in the Chinese Han population, which included 424 people with Type 2 diabetes, 878 people with impaired fasting glucose (IFG), and 1,908 people with normal fasting glucose. In this study's sample of 3,210 people, 17 single nucleotide polymorphisms were genotyped (SNPs). The SLC30A8 gene region most likely contributes to the risk of T2DM caused by -cell dysfunction, they concluded (16).

Mtiraoui et al., on the other hand, carried out studies in

2012 on Lebanese (751/918) and Tunisian (1470/838) individuals (patients/controls) to determine the risk of SLC30A8 gene on T2DM. Both populations had different associations with the SLC30A8 gene and T2DM. This study assessed the risk of developing T2DM in Lebanese and Tunisian populations (17).

Alharbi et al. conducted a genotyping study on 120 T2DM patients and 120 control groups to determine the association between the rs13266634 genetic variant of the SLC30A8 gene and T2DM disease in the Saudi population in 2021. In their ANOVA analysis, they found no relationships between the rs13266634 genotypes or the anthropometric and biochemical parameters examined in this study. Because it produced a statistically significant difference, our study was more consistent with the literature (12).

Mashal et al. genotyped 358 T2DM patients and 326 control people in 2021 to detect increased T2DM risk and SLC30A8 (rs13266634) gene polymorphism in the Jordanian population. They discovered a link between T2DM and the SLC30A8 (rs13266634) gene polymorphism as a result of their research. This study established the effect of the SLC30A8 gene polymorphism rs13266634 on T2DM disease in the Jordanian population. This study is also supported by our findings (18).

In order to establish the link between SLC30A8 gene polymorphism and T2DM, Fan et al. conducted a meta-analysis study in 2016 with data from 46 studies involving 71,890 patients and 96,753 controls. The rs13266634 C/T polymorphism was linked to a higher risk of T2DM in the allelic frequency comparisons performed after the first pooling of the general data in the meta-analysis. The findings of the study demonstrated how SLC30A8 gene variation affects Asian, European, and African populations. Although it is seen in this study that SLC30A8 gene polymorphism is effective in different ethnic origins, the fact that the study is a meta-analysis, dealing with different populations, and having a large number of studied samples gives the study an advantage. With this study, the results of our study can be said to support each other (13).

In general, this study and the studies in the literature are consistent. This adds to the significance of our work. We believe that our findings will help determine how the rs13266634 polymorphism in the SLC30A08 gene contributes to the onset of T2DM and will serve as a model for future research. The limitation of our study is that the study samples are small and limited to a specific region. Because the sample size in our study was small, we can conclude that increasing the sample size in future studies based on the evaluation of gene polymorphisms in the development of T2DM would be more beneficial.

Declaration of Interest: Regarding the publishing of this paper, the authors affirm that there are no conflicts of interest.

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The Effects of Various Dental Restorative Materials on Neuroblastoma Cells

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Abstract

Introduction: Our aim was to investigate the effects of restorative materials such as composite, compomer and glass ionomer, which are frequently used in dentistry, on SH-SY5Y neuroblastoma cells by evaluating cell viability, rate of dead/live cells, oxidative stress parameters, and pro-inflammatory cytokines. Materials and Methods: Equa Forte, Dyract AP, Tokuyama Estelite P Quick, Omnichroma, Filtek Z250, SureFil SDR flow restorative materials were used in our study. SH-SY5Y neuroblastoma cells were cultured with restorative materials. Immunofluorescence labelling was performed on the experimental groups with FDA and PI dyes. Then, ELISA technique was used to detect the levels of TNF-alpha, IL-1-beta, IL-6, SOD, LPO and CAT. One-way ANOVA analysis was used for statistical analysis ($p<0.05$). Results: In the light of the obtained data, it was observed that the dental filling materials were effective in increasing the levels/activities of all parameters including SOD, LPO, CAT, TNF-alpha, IL-1-beta, and IL-6. Immunofluorescence staining micrographs confirmed the viability analysis. Conclusion: Our study shows that biocompatibility cannot be explained by looking at a single cause. Biocompatibility varies with material content, residual monomer amount and solubility. Although all experimental groups have cytotoxic effects, the least effect is seen in the glass ionomer (Equa Forte) group.

Keywords: Capsella bursa-pastoris root, Anticandidal, Antibiofilm, Extraction, *Candida* species.

Introduction

Restorative dentistry involves restoring and maintaining oral health with appropriate restorative treatment to preserve and restore pulp function. Most of these products used in dentistry are triethylene glycol dimethacrylate (TEGDMA), ethylene glycol dimethacrylate (EGDMA), or diethylene glycol dimethacrylate (DEGDMA), B bisphenol A- glycidyl in combination with comonomers methacrylate (Bis-GMA) and urethane various methacrylates such as dimethacrylate (UDMA) (1). For this purpose, restorative materials with different properties are

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produced and there is a strong correlation between the biological compatibility with other oral tissues for the clinical success of these restorative materials (2). Dental filling materials remain in contact with the tissues in their microenvironment for a long time after use. And these materials must go through the certification process before being made available (3). The certification process also includes cytotoxicity tests for clinical risk assessments. For this reason, cytotoxicity test methods are included in international standards (4). Cytotoxicity can be evaluated by methods such as the determination of viable cell proliferation rates and enzyme activities (5).

The cytotoxicity of dental composites is tightly linked to residual monomers released due to degradation processes or incomplete polymerization of materials (6). These residual monomers, dentin dissolves in the tubules with agents such as oral fluids or other external fluids, affecting the soft tissues of the oral cavity and the dentin-pulp complex (7). In other words, as a result of the restoration, the possibility of microleakage causes negative effects on periodontal tissues (8). In light of this information, with both in vitro and in vivo studies, it has been determined that monomers have aimed to reveal their cytotoxic, genotoxic, cellular reactive oxygen production, and general health effects (9). Moreover, studies have shown that amalgam, resin composite and glass ionomers which are used as dental filling materials affect the brain by passing into the blood; however, this study is tissue-based only. However, there is no study on whether the filling materials we used in the study pass the blood-brain barrier.

In current study, our aim was to investigate the effects of restorative materials such as composite, compomer and glass ionomer, which are frequently used in dentistry, on SH-SY5Y neuroblastoma cells by evaluating the oxidative stress parameters, and pro-inflammatory cytokines.

Materials and Methods

Extract Preparation: This study was organized under the International Organization for Standardization (ISO) No: 10993-5:20097 and 10993-12:2021 standards. The experimental groups and the detailed information about dental filling materials and applicated doses are in Table 1.

Table 1. The experimental groups of our study

Group Codes	Doses	Materials	Composition	Manufacturer
Cnt	None	Control Group		
A	100 µl	Equıa Forte	Fluoroaluminosilicate glass, polybasic carboxylic acid, polyacrylic acid, water, iron oxide	GC Corporation, Tokyo, Japan
B	100 µl	Dyract AP	UDMA (Urethane dimethacrylate), Iron oxide pigments. TCB Resin (Tetracarboxylic acid-hydroxyethylmethacrylate-ester), Butyl hydroxy toluene Alkanoyl-poly-methacrylate, Strontium fluoride Strontium-fluoro-silicate glass, Photo initiators.	Dentsply De Trey, Konstanz, Germany
C	100 µl	Estelite P Quick	TEGDMA, 2-Propenoic acid, 2-methyl-, (1-methylethylidene, bis[4,1-phenyleneoxy(2-hydroxy-3,1-propanediyl)] ester, titanium dioxide, 2,6-di-tert-butyl-p-cresol; p-methoxyphenol	Tokuyama Dental Corporation, Tokyo, Japan
D	100 µl	Omnichroma	UDMA/TEGDMA Monomers, spherical SiO2-ZrO2	Tokuyama Dental Corporation, Tokyo, Japan
E	100 µl	Filtek Z250	Bis-GMA (Bisphenol glycidylmethacrylate), Non-agglomerated silica nanoparticles UDMA (urethane dimethacrylate), Bis-EMA (Ethoxylated bisphenol dimethacrylate), TEGDMA (Triethlene glycol dimethacrylate	3M ESPE, St Paul, MN, USA
F	100 µl	SureFil SDR flow	Modified UDMA, Bis-EMA, TEGDMA	Dentsply DeTrey, Konstanz,Germany

Equia Forte (GC Corporation, Tokyo, Japan) was placed in a 0.5 mm thick 6x10 cm Teflon mold after mixing in an amalgamator. Polymerization and coating application were performed according to the manufacturer's data. Compomer and composite groups (Dyract AP, Estelite P Quick, Omnichroma, Filtek Z250, and SureFil SDR flow) were also placed in a 0.5 mm thick, 6x10 mm Teflon mold and polymerized following the manufacturer's recommendations. For cytotoxicity testing, samples were placed in a 50 ml extraction flask containing 60 cm2 of test substance covered with 20 ml of minimally basic medium. After 24 hours at 37 degrees, 100 µl of solution samples were taken and applied.

Cell Culture Protocol and Cell Viability Assay: The SH-SY5Y neuroblastoma cell line was purchased from ATCC. Cells were seeded using Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic and propagated under appropriate conditions (37°C and 5% CO2). After obtaining sufficient confluence, cells were removed from the flask with the help of trypsin (Gibco, Pittsburgh, USA), centrifuged and trypsin removed. Then, the cells were stained with trypan blue (Sigma-Aldrich, USA) and the total cell number was calculated by counting under an inverted microscope.

Cell Viability Detection Kit-8 (CVDK-8) was used for cell viability analysis. For this analysis, 96-well plates were seeded with 7x103 cells per well. Then, the cells reached sufficient confluence, 100 µl of the prepared extracts were given to each well and incubated for 24 hours. After then, cells were incubated with 10 µl of CVDK-8 solution for 4 hours following the manufacturer's instruction. At the end of the period, spectrophotometric measurements were perfomed by ELISA reader (Multiskan GO, Thermo Scientific) at a wavelength of 450 nm.

Immunofluorescent Staining Technique: 24-well plates were seeded with 28x103 cells in each well and were expected to be confluent. After sufficient confluence was achieved, the drug was applied at the determined doses and incubated for 24 hours. Culture media were then removed, washed twice with PBS, fixed in a 4% formaldehyde solution for 4 minutes at room temperature. After then, formaldehyde was moved away and washed twice with PBS. For permeabilization, cells were incubated with 99.9% methanol at room temperature for 20 minutes and washed twice with PBS after incubation. Dyes prepared as 5 ml fluorescent diacetate (FDA, ThermoFisher, Cat No: F1303) and 5 ml propidium iodide (PI, ThermoFisher, Cat No: P1304MP) in 100 ml PBS were added to the wells and photographed with the help of an inverted microscope (Invitrogen Evos FL).

ELISA Technique: Cells seeded in 24-well plates were incubated with drugs for 24 hours, and the medium was collected. Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Table 2) were used following the manufacturer's instruction.

Table 2: Manufacturer's names and catalog numbers of the test kits used in this study

ELISA Kits	Producer	Catalog Number
Human IL-1-beta ELISA Kit	BT Laboratory	E0143Hu
Human IL-6 ELISA Kit	BT Laboratory	E0090Hu
Human TNF alpha	BT Laboratory	E0082Hu
Human SOD ELISA Kit	BT Laboratory	E4502Hu
Human CAT ELISA Kit	BT Laboratory	E3053Hu
Human LPO ELISA Kit	Elabscience	E-BC-K176-M

Statistical Analysis: The results were expressed as means ± SEM. Statistical significance was evaluated by one-way ANOVA followed by Tukey posttest for more than 2 independent numerical data. All data were analyzed using GraphPad Prism, version 5.0 for Windows (Graph Pad Software, San Diego, California, USA). The level of $p<0.05$ was considered statistically significant.

Results

Cell Viability Analysis Findings: Cell viability assay was performed with CVDK-8 to test whether dental filling materials (A, B, C, D, E, and F) had cytotoxic effects on SH-SY5Y neuroblastoma cell line or not. According to the results, it was detected that cell viability rates decreased in all groups (A, B, C, D, E, and F) compared to control at the end of 24 hours. The most decrease was observed in Group C concerning viability rates (approximately 53%). Following Group C, the survival rate decreased by 50%, 47% 45%, 44%, and 38% Group E, D, F, B, and A, respectively (Figure 1).

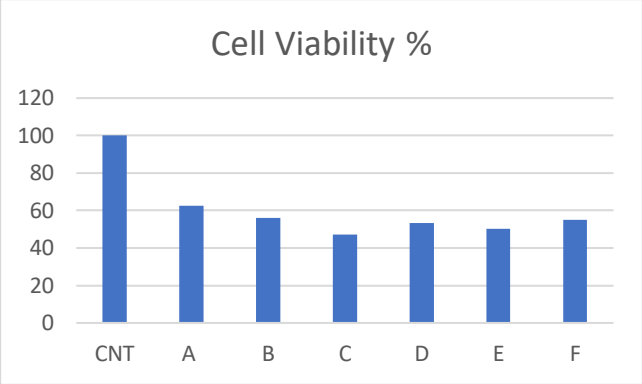

Figure 1: Cell viability test of experimental groups. The viability of the control group was accepted as 100% and the averages of the experimental groups were calculated.

Immunofluorescent Staining Results: To demonstrate the effect of dental filling materials on SH-SY5Y neuroblastoma cells, immunofluorescence labeling was performed with FDA and PI dyes. Findings were similar to cell viability results. All filling materials caused a dramatic increase in cell death. While the most dead cells were observed in Groups C and E, the least cell death was observed in Groups A and B (Figure 2).

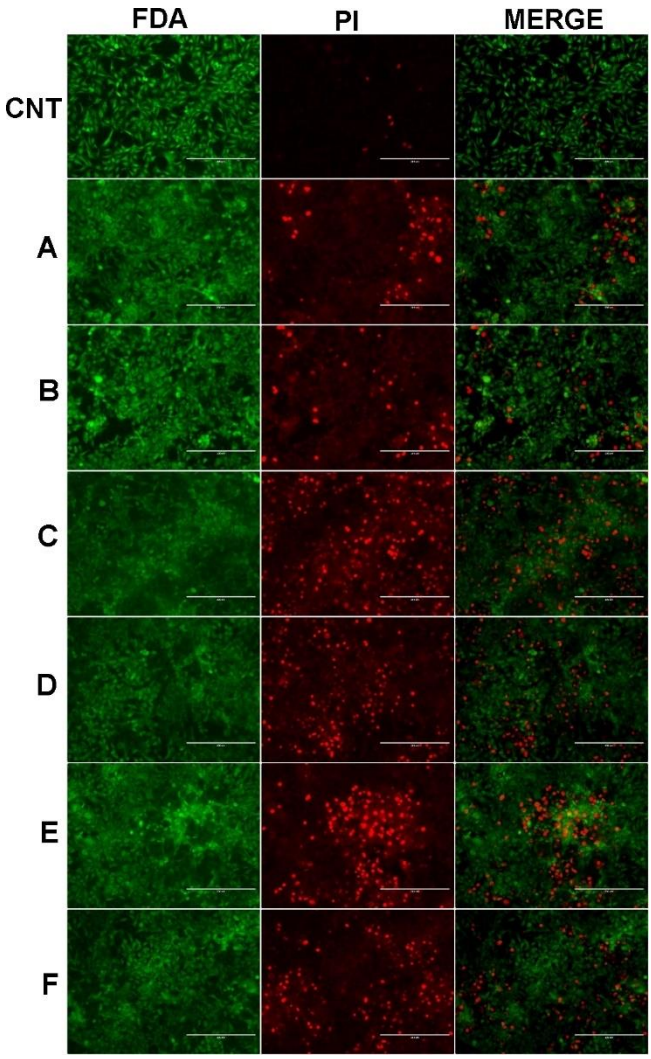

Figure 2: FDA and PI immunofluorescence labeling in experimental groups. Red cells (PI positive) indicate dead cells, and green cells (FDA positive) indicate live cells.

ELISA Results: Cell medium was collected from all experimental groups, and pro-inflammatory (TNF-Alpha, IL-1-beta, and IL-6) and oxidative stress parameters (LPO, SOD, CAT) were examined (Figure 3).

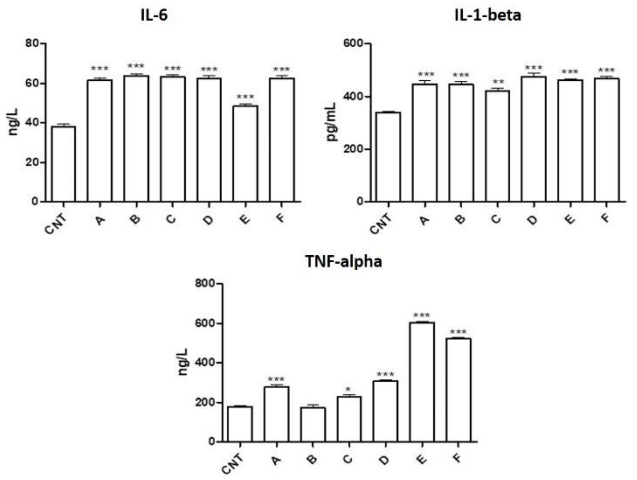

Figure 3: ELISA results of pro-inflammatory cytokines

Compared to the control group, it was determined that IL-6, IL-1-beta, and TNF-alpha levels were increased. It was observed that the highest increase in IL-6 levels was in group C, and the least increase was in group E. When IL-1-beta levels were compared, it was seen that the highest increase was in the D group and the least increase was in the C group. When the TNF-alpha levels were compared, it was observed that the highest increase was observed in the E group, while the value of the B group was very close to the control.

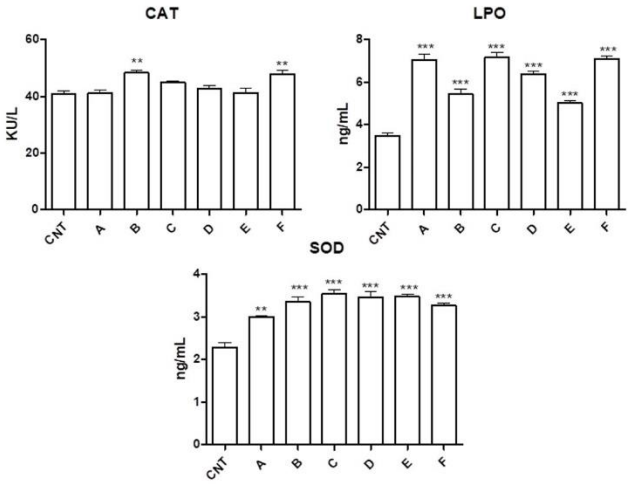

Figure 4: ELISA results of oxidative stress parameters

Compared to the control group, LPO, SOD, and CAT levels were increased. When LPO levels were compared, the greatest increase was seen in groups A, E, and D, while groups B and E were higher than the control but less than groups A, E, and D. When SOD levels were compared, the highest increase was observed in group C, and the least increase was observed in group A. When CAT levels were compared, the highest increase was seen in groups B and F, while the ratio of groups E, A, and D was close to control (Figure 4).

Discussion

Restoration of a deep cavity with a single layer of direct restorative material (more than 2.5 mm thick) has been reported to cause a significant reduction in material properties that may affect its lifespan (10). It was evaluated the effect of filler type, shade, exposure time, and cured radiant exposure on the degree of convergence of composite resins (11). Same researchers tested samples of composite resins cured through different thicknesses of already polymerized composite resins for different exposure times (20, 40, 60, and 80 s) with an irradiation level of 800 mw/cm2. They finalized that the most important factor in the degree of convergence of composite resins is thickness. A degree of convergence thickness of more than 2 mm causes significant degree of convergence lowering. Also, Rueggeberg et al. concluded that to provide an adequately polymerized composite resin it must have a 2mm rise cured for 60 seconds with irradiation levels of at least 400 mw/cm2 (12).

Flury et al. (13) tested the effect of different composite resin thicknesses on the Vickers microhardness of different composite resin types. They reported a reduction in Vickers microhardness values of conventional composite resins at a depth of more than 2 mm. In addition, Price et al. compared the effect of resin thickness on microhardness when cured with PAC or QTH lcus. The researchers reported that the thickness of composite resin has a significant effect on the hardness of composite resin. When using one of the tested lcus, only 2 mm thick specimens showed equivalent hardness values of the upper and lower surfaces at all time intervals. This indicates adequate

degree of convergence of the lower surface of the restoration (14).

Increasing the thickness of the composite resin restoration results in more curing light absorption and scattering and less light penetration within the layers of the cured material. Therefore, the overall curing light energy decreases with the increase of composite resin thickness. Accordingly, the degree of convergence value of the material decreases (15, 16). Therefore, for cavity preparation exceeding 2 mm, the incremental layering technique is considered standard for composite resin placement. It is reported that this technique allows the composite resin layers to be exposed to sufficient light and lower polymerization shrinkage (17, 18). In our study, all materials were polymerized and surface treated in accordance with the manufacturer's instructions.

Composite resin materials can be packaged according to their consistency and classified as flowable composite resins (19). Flowable composite resin has low viscosity due to the low filler level or the addition of modifiers such as surfactants (20). It is used to increase the adaptability of composite resin restoration to cavity walls and floors with very fine-tip syringes. While trying to restore the function and aesthetics of the tooth, the packable composite resin cannot be inserted into the cavity with a syringe due to its high viscosity (15, 19).

Monomer and filler type, filler content, and filler and polymer matrix refractive index all have an impact on the ability to transmit light through the composite resin layers (21). Therefore, it is reported that different composite resin compositions, filler size, weight, volume, and filler-matrix ratio have a significant effect on the degree of convergence and microhardness of composite resins (22, 23) The decrease in viability was most observed in group C (approximately 53%). When the TNF-alpha levels were compared, it was seen that the highest increase was in the E group, and the value of the B group was very close to the control. When CAT levels were compared, the highest increase was seen in groups B and F, while the ratio of groups E, A, and D was close to control.

As a summary, dental filling materials induce inflammatory response and oxidative stress in neuroblastoma cells. Current literature also support that root-end filling materials effect and increase the expression of inflammatory cytokines (24, 25). Two studies also reported that dental filling materials such as dental amalgam, glass ionomer and resin composite had a critical effect on the activities of some oxidative stress process enzymes including LPO, SOD, CAT, and GSH (26, 27).

The results of our study should be supported by performing both in vivo and in vitro tests for future studies. It has been shown that all restorative materials with different contents and chemical structures used in our study have cytotoxic effects on neuroblastoma cells. However, their potential to cause pulpal problems should be investigated by performing long-term tests.

Conclusions

Our study shows that biocompatibility cannot be explained by looking at a single reason. Biocompatibility varies according to the content of the material, the amount of residual monomer, and its solubility. Although all the experimental groups have cytotoxic effects, the least effect is seen in the glass ionomer (Equa Forte) group. More detailed studies researching the reasons of inflammatory response and oxidative stress should be performed for the biocompatibility of filling materials.

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

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Experimental hypothyroidism increases oxidative stress and apoptosis in ovary of rats

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Abstract

This study aimed to investigate hypothyroidism's effects in experimental hypothyroidism-induced female rats. Materials and Methods: To induce hypothyroidism in rats, 0.05% 6-propyl-2-thiouracil (PTU) was given orally to the hypothyroid (HT) and L-thyroxine (LT) groups with their drinking water for eight weeks. In the LT group, 0.8 µg/100 g L-thyroxine was provided by subcutaneous for ten days of the experiment. Results: In the histopathological evaluation, hypothyroidism induced severe dilation in vessels atretic and irregular follicles and damaged the germinal epithelium. These pathologies were decreased in the LT group. In the biochemical analysis, SOD activity, GSH activity, and MDA level were lower in the HT group than Control and LT groups. In the relative protein analysis, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels were lower in the Control and LT groups, but the relative protein expression of Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α was found as higher in the HT group. The protein expression of Bcl-2 level was higher in the Control and LT groups. Conclusion: The results showed that hypothyroidism causes ovarian tissue injuries —the histopathological and biochemical changes induced by Hypothyroidism in the experimental rat model were ameliorated with L-thyroxine treatment.

Keywords: Hypothyroidism, ovarian, apoptosis, oxidative stress, rat

Introduction

Thyroid hormone (TH) is an essential factor in regulati ng many biological processes, including growth, differe ntiation, metabolism, embryo development, and femal e reproduction (1-3). Altered TH levels are associated with impaired folliculogenesis, reduced fertilization rate, and reduced embryo quality in severe cases (4-7). Proper ovarian follicular development depends on the delicate balance between pituitary gonadotropic hormones and locally and peripherally produced factors. These factors include transforming growth factor beta, insulin-like growth factor, leptin, and adiponectin factors. (8-12). Active T3 (3,3', 5-

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triiodothyronine) functions as a peripheral factor by enhancing the effect of follicle-stimulating hormone (FSH), a gonadotropic hormone, on follicle growth (13). FSH and T3 exert their effects on follicular development by stimulating granulosa cell proliferation and inhibiting apoptosis through the activation of the PI3K/Akt pathway (14).

Hypothyroidism is a common health condition (15). Its prevalence increases with age and is also 5-10 times more common in women than in men (16). Although hyperthyroidism has a relatively high incidence in women, there are only a few studies that address the effects of elevated TH (thyroid hormone) concentrations in neonatal female reproductive system development, despite its overall prevalence being around 1% (17). For example, Soliman and Reineke have shown that mild thyroid stimulation in young female mice delayed the age of vaginal opening and accelerated the onset of estrus cycles compared to healthy thyroid control group. The ovaries of these hyperthyroid mice contain multiple growing follicles and corpus luteum (18).

The effects of thyroid hormone (TH) deficiency on ovarian follicular development are typically studied in animal models using propylthiouracil (PTU) as a goitrogen. However, long-term PTU treatment has been associated with various adverse effects, including agranulocytosis and hepatotoxicity (19). The administration of PTU during pregnancy often leads to preterm birth. As a result, treatment is typically initiated at the time of birth and discontinued before the offspring reach adolescence (20,21). These studies demonstrate that neonatal hypothyroidism in rats leads to a delay or even complete inhibition of vaginal opening and sexual maturation. It also results in smaller ovaries with fewer antral follicles and more atretic follicles (20).

Thyroid hormone can act as an oxidant and induce DNA damage (22). Reactive oxygen species (ROS) are continuously generated in cells as a result of both biochemical reactions and external factors (23). It is increasingly understood that ROS, when present in physiological amounts, play an important signaling role in cells (24). Indeed, regulated production of ROS is necessary for the initiation of the first meiotic division of the primary oocyte (25). This indicates that ROS serves as an important mediator during ovulation (24,26). Reducing ROS production is necessary during the secondary meiotic division, demonstrating the need for a balance between ROS production and cellular ROS defense. Disruption of cellular antioxidant defenses, especially with mitochondria playing a significant role as a source, can lead to oxidative stress and associated pathologies (27).

It is not clear that chronic hypothyroidism affects the expression of proteins associated with ovarian redox balance and cell survival. The reduced plasma TH concentration affects the ovarian follicular reserve and ovulation. We believe that there is a relationship between oxidative stress and ovarian reserve. To evaluate this hypothesis, the relative expression of Bcl-2, Caspase-3, NF-κB, mTOR, P2X7R, IL-1, TNF-alpha, mTOR, and oxidative parameters will be examined at the end of the experiment.

Materials and Methods

Animal Procedure: Ethical approval for the study was obtained from the Ataturk University Animal Experiments Local Ethics Committee. A total of 36 twelve-week-old Sprague-Dawley female rats, weighing between 200 and 250 grams, were procured from the Ataturk University Experimental Research and Application Center. Before commencing the experiments, the animals were acclimated to their environment and then divided into groups. Throughout the study, the rats were provided with water and pellet food ad libitum. The animals were kept in laboratory conditions with a 12-hour light/dark cycle, at a temperature of 21-23°C, and a relative humidity of 45-50% during the experiments.

Animal Grouping: For the experiment, three groups were formed, each consisting of 12 rats. The initial weight measurements of the animals were conducted on the day the experiment commenced, and subsequently, they were weighed at consistent weekly intervals throughout the study.

Experimental groups: No drug treatment was administered to the Control group. In the Hypothyroid (HT) and Treatment groups (LT), a 0.05% PTU (6-propyl-2-thiouracil) (Sigma, P3755) solution was freshly prepared daily and administered by mixing it into the drinking water for eight weeks to induce a hypothyroid model (28,29). Treatment Group (LT): The rats were administered 0.05% PTU, freshly prepared daily, by mixing it into their drinking water for a duration of eight weeks. Once the hypothyroidism model was successfully induced, the rats received a subcutaneous daily dose of 0.8µg/100g L-thyroxine (Santa Cruz, sc-207813A) for ten consecutive days (28).

Surgical Procedures: To conclude the experiment, the animals were intraperitoneally anesthetized with 100 mg/kg ketamine and 15 mg/kg xylazine. They were euthanized by decapitation, and ovarian tissues were then extracted. Tissues designated for histopathological examination were promptly immersed in a 10% formaldehyde solution.

Histopathological Analysis: The obtained ovarian tissues were fixed in 10% naturally buffered formaldehyde solution for 24 hours, then passed through a graded alcohol series, cleaned with xylene, and embedded in paraffin blocks. Blocks were cut to a thickness of 5 µm and stained with Crossman Modified Triple staining for histopathological evaluation. The slides were evaluated by photography with a Trinocular Microscope (Zeiss, Axio1, German).

Biochemical Analyzes in Ovarian Tissues: After the experiment was terminated, ovarian tissue samples taken from rats in each group (n: 6) were ground using the TissueLyser II grinding set (Qiagen, Hilden, Germany). 50 mg of each of the tissue samples ground into 2mL Eppendorf tubes will be taken and homogenized in 1 mL of phosphate buffer (100 mM, pH 7.0) using the TissueLyser II grinding set (Qiagen, Hilden, Germany). After the homogenization process, the samples will be centrifuged, and superoxide dismutase (SOD) activity, lipid peroxidation levels (LPO), and glutathione (GSH) levels will be measured from the supernatant sections.

Measurement of Superoxide Dismutase Enzyme Activity in Ovarian Tissues: Superoxide dismutase (SOD) activity in ovarian tissue tissues was determined by Sun et al (30). According to the protocol of this experiment, 50 mg of tissue was homogenized with 1 mL phosphate buffer (0.1 mM, pH 3) and then centrifuged at 18000xg for 60 min. After pipetting 500 µL supernatant and 2450 µL measurement mixture (0.3 mM xanthine, 0.6 mM EDTA, 150 µM NBT, 0.4 M Na_2CO_3, 1.2 g/L BSA) and 50 µl xanthine oxidase into the tubes prepared for each sample, from the supernatant obtained after centrifugation. Then it was incubated for 20 minutes. At the end of the incubation period, 100 µL $CuCl_2$ (0.8 mM) was pipetted to stop the reaction. The absorbance of the formazone dye formed due to the reaction was measured at 560 nm. All samples were studied in three replicates, and the results were calculated from the prepared SOD standard chart and expressed as nM/minute/mg tissue.

Measurement of Total Glutathione (GSH) Levels in Ovarian Tissues: Total glutathione level in ovarian tissues was determined using a modified version of the method developed by Sedlak and Lindsay (1968)(31). According to the protocol of this experiment, 50 mg of tissue was homogenized with 20 mM EDTA, 1 mL Tris-HCl (50 mM, pH 7.4), and then centrifuged at 12000xg for 4 min. From the supernatant obtained after centrifugation, 500 µL supernatant, 1500 µL Tris-HCl (200 mM, pH 8.2), 100

µL DTNB, and 7900 µL methanol were pipetted into the capped tubes prepared for each sample. After pipetting, they were incubated at 37 °C for 30 minutes, and then their absorbance was measured at 412 nm. All samples were studied in three replicates, and the results were calculated from the prepared GSH standard graph and expressed as nmol/g tissue.

Measurement of Lipid Peroxidation (LPO) Levels in Ovarian Tissues: Lipid peroxidation level in ovarian tissues was determined by Ohkawa et al(32). According to the protocol of this experiment, 50 mg of tissue will be homogenized with 4.5 mL KCl (10%). After the homogenization process, it was centrifuged at 5000xg for 20 minutes. From the supernatant obtained after centrifugation, 250 µL supernatant, 100 µL sodium lauryl sulfate (8%), 750 µL acetic acid (20%), 750 µL TBA (0.8%), and 150 µL distilled water were added into the capped tubes prepared for each sample. Pipetted. Following the pipetting process, it was mixed with the help of a vortex and incubated in a shaking water bath at 100 °C for 60 minutes. At the end of the incubation period, the sample tubes were cooled to room temperature under tap water. After cooling, 2.5 mL of n-butanol: pyridine (15:1) was pipetted and then centrifuged at 4000xg for 30 min, and then the absorbance was measured at 532 nm. All samples were studied in three replicates, and the results were calculated from the prepared MDA standard chart and expressed as nmol/g tissue.

Western Blot Analysis: The tissue samples were homogenized using a tissue lyser device (Qiagen, USA) at 30 Hz for 30 sec to the extraction of proteins for western blot analysis after being weighted and crushed in nitrogen gas, treated with radioimmunoprecipitation (RIPA buffer, Ecotech Bio, Turkey), supplemented with protease and phosphatase inhibitors, and treated with protease and phosphatase inhibitors. The relative protein expressions of Bcl-2, Caspase-3, P2X7R, mTOR, IL-1β, and TNF-α were then determined by quantifying the proteins using a commercial kit (Pierce BCA, Thermo Sci., USA). The protein loaded membranes were incubated at 4 °C overnight with the appropriate primary antibodies ((IL-1β (sc-52012, Santa Cruz), TNF-α (sc-52746, Santa Cruz), P2X7R antibody (11144-1-AP, Proteintech), Caspase-3 (sc-56053, Santa Cruz), Bcl-2 (sc-7382, Santa Cruz), NfkB-p65 (AF5006, Affinity biotech) and mTOR (AF6308, Affinity Biotech), and Beta-actin (sc-47778, Santa Cruz). secondary antibody. After primary antibody incubation, the PVDF membranes were washed with TBST and then incubated for 90 minutes at room temperature with the second antibody (Santa Cruz, sc-2004/sc-2005) coupled to horseradish peroxidase. Then, the protein bands were captured using the enhanced chemiluminescence reagent Western ECL substrate (Thermo, 3405), visualized, and analyzed by Image Lab™ Software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis: The analyses were performed depending on whether the data were parametric or not. While the Shapiro-Wilk test was used to determine the normality of data distributions, the homogeneity of variance of the groups was determined by the Levene test. One-way analysis of variance was used for data that met parametric assumptions, and Mann Whitney U test was used for multiple comparisons if the Kruskal Wallis test was significant for data that did not meet parametric assumptions. SPSS (IBM SPSS Statistics Desktop 20.0) program was used while performing the tests.

Results

Histopathological Evaluations: In the histopathological examination performed in the control group, follicles and corpus luteum of different sizes and periods were detected in the cortex and were observed to be normal. The germinal epithelium was normal and regular (Figure 1 a). In the hypothyroid group, it was observed that the vessels were severely

dilated, there were many atretic and irregular follicles, and the germinal epithelium was irregular and damaged (Figure 1 b). In the treatment group, it was observed that atretic and irregular follicles decreased, the deterioration in vascular structures was less, and the germinal epithelium was regular (Figure 1c).

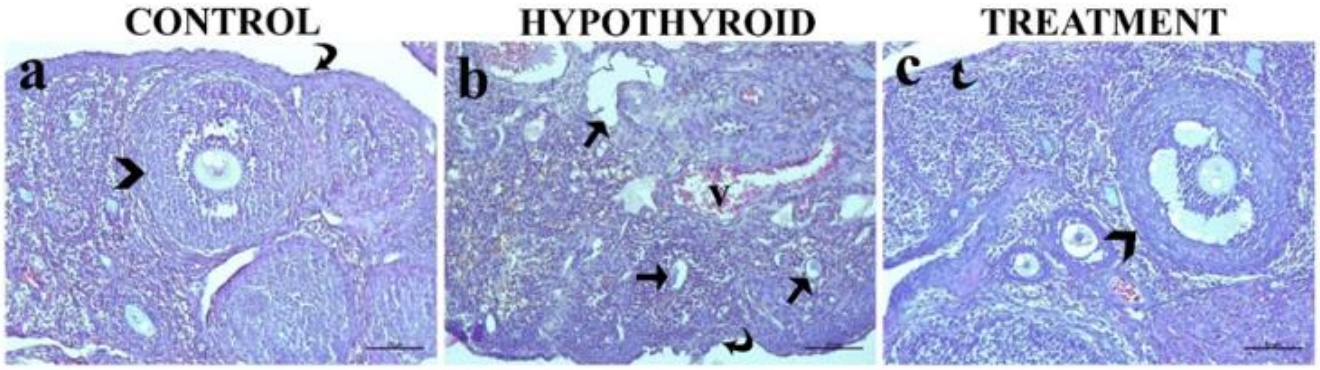

Figure 1. Illustration of histologic ovarian section all groups. Arrow head; secondary follicle, curved arrow; germinal epithelium, Arrow; atretic follicle, v; dilated vein. Crossman Modified Triple Staining, Magnification 200X.

When the numbers of antral follicles were compared among the three groups, a significant difference was observed (Table 1).

Table 1. Antral follicle count by group in sections evaluated after H&E staining.

		Left Ovary	Right Ovary
		Antral follicle	Antral follicle
CONTROL	M ±SD	6,71±1,25	5,14±1,77
LT	M ±SD	3,43±0,98	2,85±1,07
HT	M ±SD	2,14±0,81	1,28±0,95
P-VALUE	M ±SD	0,001	0,001

Mean (M), Standard Deviation (SD)

Biochemical Results: In the ovarian tissue biochemical analysis, the SOD activity was higher in the Control and LT groups but dramatically decreased in the HT group ($p<0.05$). GSH levels were higher in the Control and LT groups but slightly decreased in the HT groups ($p>0.05$). Tissue MDA level was higher in the HT group but significantly reduced in the Control and LT groups ($p<0.05$). The levels and comparisons of tissue biochemical analysis are presented in Table 2.

Table 2. Tissue biochemical analysis results for all groups: Superoxide dismutase (SOD), Glutathione (GSH), and Malondialdehyde (MDA).

	Sod Activity (U/Ml)	Gsh Activity (Mm/ Mg Protein)	Mda Level (µm/Mg Protein)
CONTROL	21.44±4.23^{a}	7,05±2.15^{a}	2,11±0.56^{a}
LT	19,33±3.21^{a}	6,60±2.45^{a}	2,96±0.44^{a}
HT	7,71±1.35^{b}	5,55±1.43^{a}	3,55±0.77^{b}

The letters (a,b) indicates the statistical differences among the groups, $p<0.05$ accepted.

Western Blot Results: In the analysis of relative protein expressions, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels lower in the Control and LT groups, but the relative protein expression of Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α were found as higher in the HT group. The protein expression of Bcl-2 level was found higher in the Control and LT groups but increased in the HT group. The bands and levels of relative protein expressions for all groups are presented in the Figure 2.

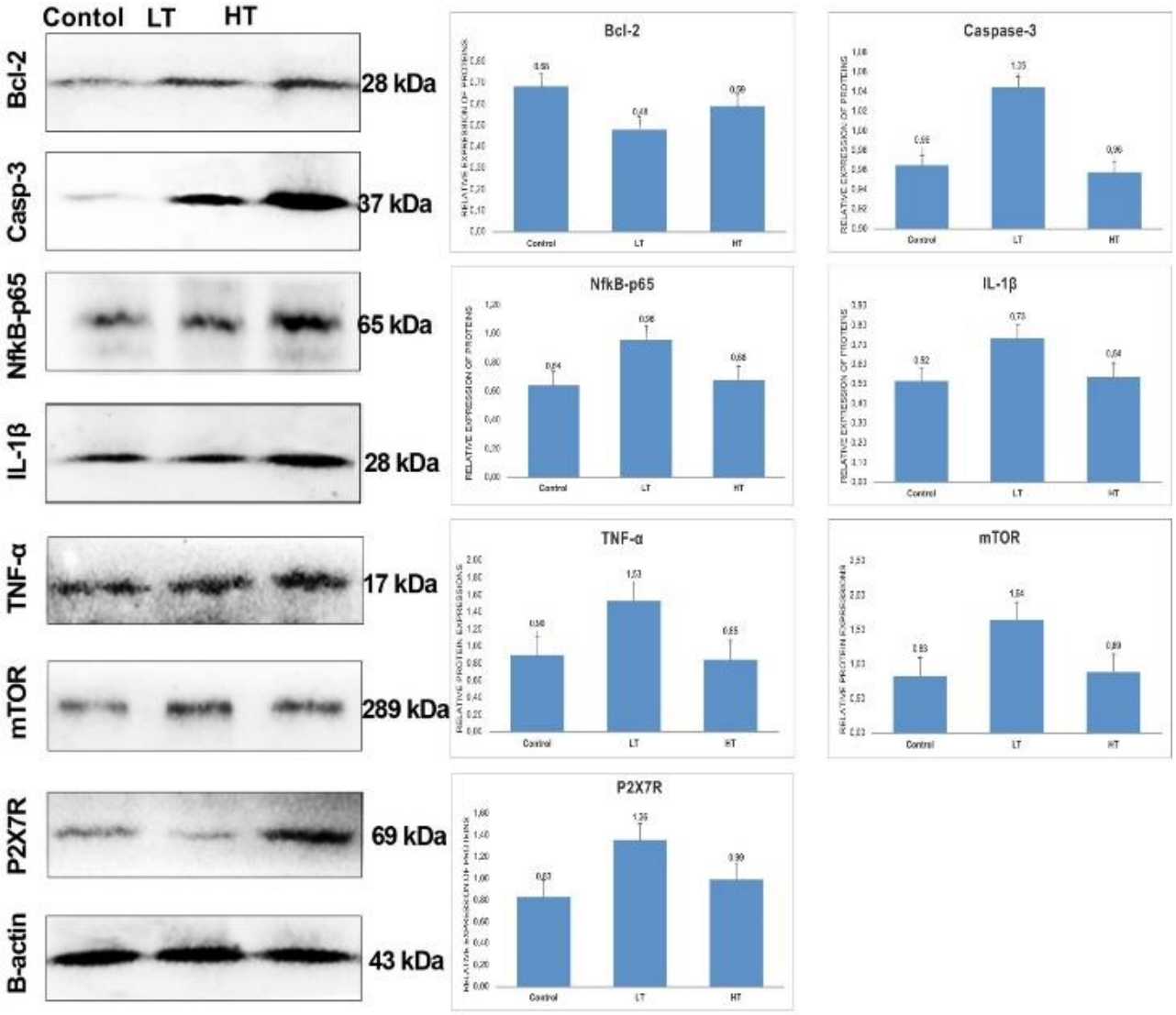

Figure 2. The analysis of relative protein expressions, Caspase-3, mTOR, P2X7R, NfkB, IL-1β, and TNF-α levels.

Discussion

In this study, ovarian tissue damage, oxidative stress level and apoptotic activity were analyzed in detail in adult female rats exposed to chronic hypothyroid conditions for a long time. In hypothyroid ovaries, tissue damage, decreased follicle reserve, and oxidative parameters and apoptotic activity increased significantly compared to controls. Decreased antioxidant levels and increased apoptotic activity in the hypothyroid group probably lead to a decrease in follicle reserve through cellular damage.

When experimental hypothyroidism is induced in female rats, degenerative changes occur in their ovaries, and the estrous cycle is altered (33). In the conducted studies, untreated mature hypothyroid rats were observed to have no signs of estrus and no ovulation. Histological examination showed that most follicles were atretic, and corpus luteum was not observed. Gonadotropin treatment triggered ovulation, but the number of eggs ovulated was lower than that in normal control groups. These findings were consistent with the results of previous studies on hypothyroid rats and chickens, demonstrating that hypothyroidism inhibits follicular development and ovulation (34,35). In the ovaries of animals treated with PTU, compared to rats not treated with PTU, there were more

secondary follicles and fewer antral follicles, while non-atretic antral follicles were smaller, and the number of atretic follicles was higher. However, corpus luteum was not observed. This indicates that folliculogenesis is disrupted, and this disruption is related to the diameters of antral follicles (20). In another study aiming to investigate the effects of long-term hypothyroidism on ovarian follicle reserve in adult rats, the total number of follicles in each ovarian was determined. Hypothyroid females showed an approximate 60% decrease in primordial follicle count compared to age-matched control groups after 16 weeks of hypothyroidism. Additionally, a roughly 40% reduction in primary and preantral follicle counts compared to control groups was observed in hypothyroid rats, and there was a noticeable decreasing trend in antral follicle counts, although this decrease was not statistically significant. Consistent with these observations, there was a decreasing trend in the total number of Corpus Luteum (CL) per ovarian in hypothyroid females, but this decrease was also found not to be statistically significant (36). The findings observed in the hypothyroid group were quite pronounced. In this group, there was a significant dilation of blood vessels and the presence of numerous atretic follicles and irregularly shaped follicles. Additionally, the germinal epithelium was irregular and damaged. These results appear to be in line with other studies in the literature. Previous research has also demonstrated that hypothyroidism leads to similar changes in ovarian tissue and has adverse effects on follicular development. Therefore, the findings of our study contribute to the broader context of evaluating the effects of hypothyroidism on ovarian tissue and demonstrate consistency with previous research.

It is known that antioxidant levels in the serum can decrease in the case of hypothyroidism. This decrease has been observed to weaken the body's protective abilities against reactive oxygen species (ROS) and increase oxidative stress (37). Specifically, hypothyroid individuals have been observed to have low levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and thioredoxin reductase (Txnrd1) (38). It is known that excessive TSH directly increases oxidative stress (OS) (39). Other studies have confirmed increased lipid peroxidation in overt hypothyroidism and subclinical hypothyroidism, demonstrated by elevated levels of MDA. Protein oxidation has also been reported, with increased levels of protein carbonyls observed (40). The correlation analysis conducted in this study suggests that both the increase in TSH and the elevation in MDA contribute to protein damage. Finally, various studies have reported increased levels of NO (41,42). In the biochemical analysis of ovarian tissue, SOD activity was higher in the Control and LT groups but dramatically decreased in the HT group. GSH levels were higher in the Control and LT groups but showed a slight decrease in the HT group. Tissue MDA levels were higher in the HT group but significantly decreased in the Control and LT groups. These findings appear to be consistent with similar studies in the literature and support that hypothyroidism leads to increased oxidative stress and reduced antioxidant activity in ovarian tissue.

This study, by showing increased IL-1β levels associated with subclinical hypothyroidism (SH), a subclinical form of hypothyroidism, has obtained results consistent with similar findings in the literature. This suggests that hypothyroidism may increase inflammation markers and emphasizes the need for further research in this area. In conclusion, increased serum IL-1β levels were observed in rats with SH (43). In our study, levels of Il-1β and TNF-α, which are inflammation parameters, were high in the hypothyroid group.

The results of this study conducted on ovarian tissue are consistent with previous research on hypothyroidism and Bcl-2 in the literature. Other studies in the literature have suggested that

hypothyroidism can affect Bcl-2 gene expression and that this effect can regulate cellular apoptosis. Therefore, the findings of this study confirm the impact of hypothyroidism on Bcl-2 in line with these literature data. In the hypothyroidism model created with methimazole, an increased autophagic process has been detected in the cerebellum. Additionally, it has been observed that the levels of p-AKT/AKT, p-mTOR/mTOR, and p-ULK1/ULK1 in the cerebellum have decreased due to hypothyroidism (44). In the same study, it was also observed that hypothyroidism led to an increase in c-Caspase-3 and TUNEL-positive (apoptotic) cell counts in cerebellar neurons (44). Our results show that while Caspase, NFKB, and mTOR protein expressions were increased in the hypothyroid group, the increase in apoptotic activity is compatible with findings previously reported in the literature.

In light of the results, this study shows that long-term mild hypothyroidism causes ovarian damage, oxidative stress, inflammation, and apoptosis, which may negatively affect fertility.

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

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Author Contribution: SG and NAC contributed to the conception and design of the study. SG organized the database. SNP and EA performed the statistical analysis. NAC wrote the first draft of the manuscript. NAC, SNP, and EA wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Biotic potential of aphid parasitoid, Diaeretiella rapae (Hymenoptera: Braconidae: Aphidiinae) at different temperature regimes

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Abstract

The efficiency and development of the insect's natural enemies affects greatly with changes in environmental conditions. Diaeretiella rapae M'Intosh (Hymenoptera: Aphidiidae) is one of the most common and successful parasitoids of the cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). The biotic potential of D. rapae fed on B. brassicae was assessed in terms of parasitism rate, adult emergence, longevity and developmental period at different temperature regimes; 15, 20, 25, and 30 ºC. Results of the present research showed that maximum percent parasitism (41.16% ± 0.80) and adult emergence (75.43% ± 1.04) of D. rapae were observed at 25ºC and minimum at 15ºC of about 14.02% ± 0.97 and 36.72% ± 1.93 respectively. Total longevity and development period (egg-adult emergence) at higher temperature (30ºC) were observed about 6.33 ± 0.33 days and 7.67 ± 0.57 days respectively. Adult longevity of D. rapae was maximum at 25ºC of about 9.67 days ± 0.34. Development time of parasitoid was higher (17.33 days ± 1.16) at lower temperature regime (15ºC). The study indicates that development between 20-25oC was favorable for D. rapae parasitoid and it can tolerate to a higher temperature at 30oC. Therefore, it could be a preferable candidate for the biocontrol program for aphids in relatively warmer climates.

Keywords: Development time; *Diaeretiella rapae*; *Brevicoryne brassicae*, Parasitism; Threshold temperature.

Introduction

The cabbage aphid, Brevicoryne brassicae (L.) (Hemiptera: Aphididae) is a herbivorous perennial insect pest species attacks many cruciferous crops (1). It causes direct damage by resulting chlorosis and leaf curling, while disrupting the plant growth and development (2). It also causes indirect damage through transmission of viral diseases (3). Nowadays, biological control is being considered a satisfactory tool in integrated pest management (IPM). For the eco-

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friendly management of various insect pests, parasitoids have been applied successfully in the cropping system (4). Diaeretiella rapae M'Intosh (Hymenoptera: Aphidiidae) is a cosmopolitan and koinobiont endoparasitoid of many aphid species (5) and is being considered as a successful parasitoid of B. brassicae (6). D. rapae parasitizes more than 60 aphid species on a range of horticultural and agricultural insect pests (7). Parasitic attributes of many parasitoids are impacted by environmental conditions(8). Ambient temperatures are important to determine population dynamics of parasitoids and their dispersal in suitable habitats (9). The parasitism rates mainly depend on the ability of any parasitoid to locate the host successfully, select, and oviposit in or on the hosts. All these processes are often temperature dependent, so environmental variations may affect the interactions between the host and the parasitoid by altering virulence in parasitoids and resistance in the host (10). Temperature changes various biological aspects of insects such as sex ratio fertility, survival, adult longevity (11). Thermal low and constant temperature gives valuable information about the development rate of certain arthropods (12). However, the parasitoid's potential, adult longevity, development time, total life span and parasitism rate depend on the temperature (13).

The present study was performed to evaluate the biotic potential including parasitism rate, adult emergence, longevity and development period of D. rapae when fed on B. brassicae under different temperature regimes.

Materials and Methods

From reared culture one month old (2nd generation) of B. brassicae and newly emerged adult parasitoids were used for this experiment.

Experimental procedure: Colonies of about hundred aphids (both adult and nymph) were released on fresh twigs of canola plants into plastic cages (30×25×10 cm). Five pairs of parasitoids were introduced for twenty-four hours stinging period. At the end of oviposition period, the plants were exposed nymph were transfered into clean petridishes and placed in incubator at different temperature regimes (15, 20, 25 and 30 ºC and relative humidity for all temperature regimes was same 65-70 %). Aphid population was observed four times a day to check mummies formation. Mummies were collected in glass vials and reverted to same temperature. All mummies were observed daily until emergence of adult parasitoids. Adult parasitoids were transfered into plastic vials (25 × 10 cm) and kept under the same temperature. Adult were fed with 10% honey solution (14).

Study parameters, Percent parasitism: After mummies formation, percent parasitism was recorded by using by this formu

$$\text{Percent parasitism} = \frac{\text{No. of mummies}}{\text{Total number of aphids}}\ x\ 100$$

Percent emergence: For percent emergence of adult parasitoid, each mummy was put into Eppendorf tube. When mummies change into dark brown (near to emergence), a cotton soaked honey solution (9:1) was kept into tube (15). Percent emergence was recorded by using this formula,

$$\text{Percent emergence} = \frac{\text{No. of adult emerged}}{\text{Total number of mummies}}\ x\ 10$$

Adult longevity: To calculate adult longevity of D. rapae from each aphid density, emerged parasitoids were transfered into clean Eppendorf tube. Parasitoids were fed by 10 % honey solution. Daily observation was done to check the longevity until they died.

Development periods (Egg laying to adult emergence) at different temperature regimes were also recorded.

Statistical analysis: Data on percent parasitism, adult emergence and adult longevity of parasitoid were analyzed using completely randomized design (CRD) to

check the effect of different temperature regimes on parasitoid and means were separated by tukey's HSD all pair-wise comparison test.

Result

Analysis of variance for percent parasitism and adult emergence of Diaeretiella rapae at different temperature regimes: Analysis of variance for percent parasitism of the D. rapae and adult emergence of parasitoid at different temperature regimes under completely randomized design are shown in (Table 1). The result showed that temperature regimes as treatment had highly significant impact on the percentage parasitism and adult emergence of D. rapae. Because P-value is <0.001 for both analysis of variance.

Table 1 Analysis of variance for percent parasitism and adult emergence of *D. rapae* at different temperature regimes

SOV	DF	SS	MS	F	P
Percentage parasitism					
Treatments	3	1202.65	400.883	99.9	<0.001
Error	8	32.09	4.011		
Total	11	1234.74			
Percent emergence					
Treatments	3	2413.06	804.355	12.6	<0.001
Error	8	509.91	63.738		
Total	11	2922.97			

(P≤0.05) Highly Significant

Percentage parasitism of Diaeretiella rapae at different temperature regimes against Brevicoryne brassicae: Percent parasitism of D. rapae at different temperature regimes against aphid host, B. brassicae is shown in (Fig 1). The result showed that percent parasitism increased with the increased in temperature. But the parasitoid decreased parasitism rate when temperature is more than its capacity to parasitized due to the excessive temperature. This graph showed that maximum percent parasitism (41.16 % ± 0.80) was observed under 25 ºC and minimum parasitism (14.02 % ± 0.97) was recorded at 15 ºC of D. rapae. Other two temperature regimes, 20 ºC and 30 ºC showed the percent parasitism of 34.57 % ± 1.47 and 30.51 % ± 1.27 respectively. This result showed that temperature (20-25 ºC) was most suitable for parasitism under laboratory conditions.

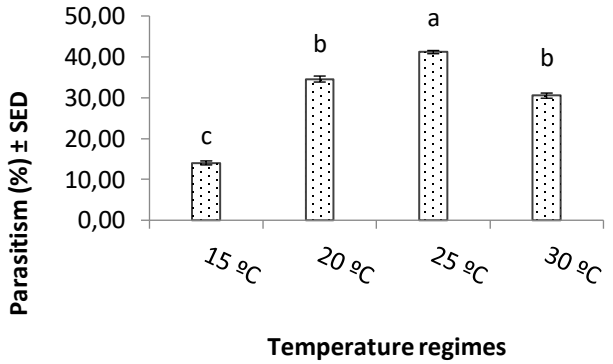

Figure 1. Percentage parasitism of D. rapae at different temperature regimes against *B. Brassicae* (Mean ± SED) mean followed by same letters are not significantly different for each temperature regime (Tuckey HSD, P≤0.05)

Adult emergence of Diaeretiella rapae from mummies at different temperature regimes against Brevicoryne brassicae: Diaeretiella rapae adult emergence from mummies at different temperature regimes is shown in (Fig. 2). The results showed that increased in temperature regimes the percent adult emergence from mummies of B. brassicae leads to increase. The maximum percent adult emergence (75.43 % ± 1.04) was recorded at 25 ºC and minimum adult emergence (36.72 % ± 1.93) of D. rapae at 15 ºC. Other two temperature regimes showed 49.17 % ± 2.04 and 59.35 % ± 1.35 adult emergence of D. rapae at 20 ºC and 30 ºC respectively.

All the temperature regimes used in present findings were significantly different to each other. But most preferable temperature for adult emergence of D. rapae from B. brassicae was 20-25 ºC.

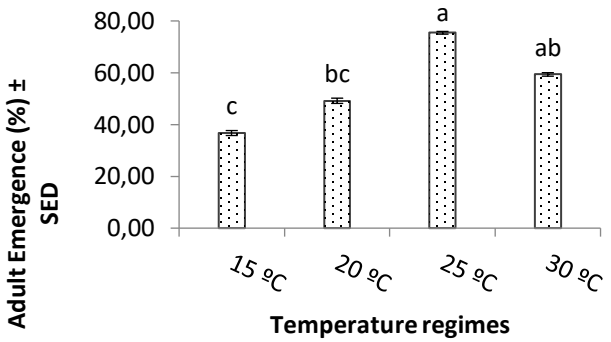

Figure. 2. Adult emergence of Diaeretiella rapae from mummies at different temperature regimes against Brevicoryne brassicae (Mean ± SEM) mean followed by same letters are not significantly different for each temperature regime (Tuckey HSD, P≤0.05)

Relationship of different temperature regimes with percent parasitism and adult emergence of *Diaeretiella rapae:* Relationship of different temperature regimes with percent parasitism and adult emergence of D. rapae from mummies of B. brassicae are shown in (Fig. 3). The present findings showed that with the increase in temperature the percent parasitism and adult emergence are also increases. The R2 value for percent parasitism and percent adult emergence are 0.3918 and 0.551 respectively. The linear trend line shows the positive relationship of temperature regimes with parasitism and emergence. But from its values and trend line it clearly shown that percent adult emergence showed strong relation instead of percent parasitism which showed weak relation with temperature regimes.

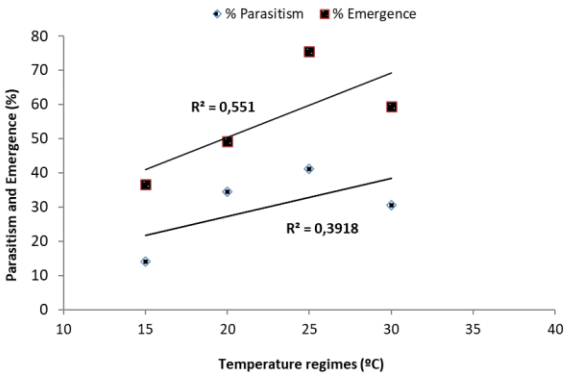

Figure 3. Relationship of different temperature regimes with percent parasitism and adult emergence of D. rapae

Total longevity and development time of Diaeretiella rapae at different temperature regimes: In (Table 2) total longevity and development (Egg-Emergence) of D. rapae at different temperature regimes are shown in this table. In case of total longevity of D. rapae at different temperature regimes, the maximum longevity (9.67 days) was recorded at 25 ºC and minimum longevity (6.33 days) was recorded at 30 ºC. Other two temperature regimes which are used in present studies showed 6.67 and 8.00 days at 15 ºC and 20 ºC respectively. The results showed that different temperature regimes strongly impact on adult longevity. Same results were noticed in case of development period of D. rapae at different regimes of temperature. The maximum development period (17.33 days) was recorded at 15 ºC temperature and minimum (7.67 days) at 30 ºC temperature. The results clearly shown that temperature increases lead to increase in total adult longevity and development period from egg laying to adult emergence. But due to maximum (30 ºC) and minimum (15 ºC) temperature the total longevity and development time period increased as well decreased. Because at low temperature parasitoid survival is minimum in case of average temperature. The best temperature range for development at longevity is 20-25 ºC in laboratory condition.

Table: 2 Total longevity and development time of D. rapae at different temperature Regimes

Temperature Regimes (ºC)	Total longevity (Days) ± SE	Development (Days) ± SD
15	6.67 ± 0.67[b]	17.33 ± 1.16[a]
20	8.00 ± 0.58a[b]	14.66 ± 0.58[b]
25	9.67 ± 0.33[a]	10.33 ± 0.57[c]
30	6.33 ± 0.33[b]	7.67 ± 0.57[d]

ANOVA values for total longevity showed F= 9.19 and P= <0.005 and for development F= 96.2 and P= <0.001

Discussion

In biological control, natural enemies are unprotected from the ecological interaction such as temperature which change the biological parameters (16) Diaeretiella rapae is most popular among different other parasitoids. The efficacy of parasitoid, D. rapae is significantly temperature dependent. Their adult longevity, parasitism, adult emergence, and development time depend upon temperature. To check these parameters present study was conducted under laboratory. Result of this study showed that the percent parasitism increased with increased in temperature, but very high temperature negatively impacts on level of parasitoid potential. Present findings were that maximum percent parasitism (41.16 %) was observed under 25 ºC and minimum parasitism (14.02 %) was recorded at 15 ºC of D. rapae. Other two temperature regimes, 20 ºC and 30 ºC showed the percent parasitism of 34.57 % and 30.51% respectively. This result showed that temperature (20-25 ºC) is most suitable for parasitism under laboratory conditions. Present findings confirmed with Fand et al. (2011) and Abdin et al. (2013) that due to changing in environmental condition the fitness of parasitoid affected. These finding similar to Sigsgaard (2000); Malina and Praslicka (2008) who reported that percentage parasitism of parasitoid increased with increased temperature. According to studies of Tahriri et al. (2007) and Appiah et al. (2013) the temperature showed significant impact on parasitism rate of parasitoid wasps. Present results are also correlate with the Zamani et al. (2007) and Pourtaghi et al. (2016) who concluded that parasitoids, A. colemani and A. matricariae showed maximum parasitism at 25 ºC against A. gossypii. But due to increase in temperature (30 ºC) the mortality rate of both parasitoids increased. Present results suggested that decrease in parasitization at 30 ºC the parasitoid D. rapae cannot tolerate especially in month of summers.

Present findings resulted that R2 value for percent parasitism and percent adult emergence are 0.3918 and 0.551 respectively. The linear trend line shows the positive relationship of temperature regimes with parasitism and emergence. But from its values and trend line it clearly shown that percent adult emergence showed strong relation instead of percent parasitism which showed weak relation with temperature regimes. These findings also similar to Sigsgaard (2000) who showed that there is linear relationship was present between parasitism and temperature. So, it recommended that at higher temperature the parasitoid could not perform well.

In case of adult longevity, the results showed that D. rapae at different temperature regimes, the maximum longevity (9.67 days) was recorded at 25 ºC and minimum longevity (6.33 days) was recorded at 30 ºC. Other two temperature regimes which are used in present studies showed 6.67 and 8.00 days at 15 ºC and 20 ºC respectively. The results showed that different temperature regimes strongly impact on adult longevity. When physical factors such as temperature

and relative humidity are maximum the fecundity rate affected the longevity of parasitoid. These present findings are correlated with the Miller and Gerth (1994) who observed that higher temperature negatively impact on adult longevity of A. matricariae. They also suggested that at higher temperature there is less emergence of adult parasitoid occur. These results are also same with present findings results. Same results are concluded by Van Tol and Van Steenis (1994) and Ohta et al. (2001) that A. colemani survival rate of decreased with increased in temperature and same effect present on A. gefuensis respectively. These results can be helpful for determination of stage of parasitoid which released in field level for biological control.

The results of present findings about development period of D. rapae showed that development period of D. rapae at different regimes of temperature. The maximum development period (17.33 days) was recorded at 15 ºC temperature and minimum (7.67 days) at 30 ºC temperature. The results clearly shown that temperature increases lead to increase in development period from egg laying to adult emergence. But due to maximum (30 ºC) and minimum (15 ºC) temperature the total longevity and development time period increased as well decreased. Because at low temperature parasitoid survival is minimum in case of average temperature. These results are also correlate with Malina and Praslicka (2008) who observed that development period for D. rapae under laboratory conditions are 8-15 days at 15 - 25 ºC, but at 30 ºC was lower (11 days). Results of present findings are similar to the results of (8) who observed that minimum development period (10.6 days) at 30 ºC and maximum period (24.3 days) at 15 ºC. They suggested that development period decreased with increase in temperature.

At higher temperature the metabolic rate of foraging insect increased with increased in temperature according to Wu et al. (2011). My results are similar to the results of (13) who observed that higher temperature increases the metabolic rate, parasitism rate and as well as egg laying capacity of parasitoid increased. According to Mohyuddin and Greathead, 1970; Overholt et al., 1997; Potting, 1997 and Wiedenmann et al., 1992) the interaction of relative humidity and temperature positively affect the adult longevity of C. flavepes. Results of this experiments are similar with Hayakawa et al., (2014) who investigated that reproductive potential and development period of D. rapae are maximum at 30 ºC, but at high temperature the longevity of parasitoid reduced which ultimately reduced the fecundity rate. The present findings show same results as Moayeri et al., (2013) who examined that searching rate and handling time are influenced by the high temperature. Present results are also similar with Goh et al., (2001) who reported that A. colemani population are influenced with temperature. The consequences of present research might be useful for mass rearing as well as release of D. rapae in field for the control of B. brassicae. Further experiments will also be conducted to investigate the role of temperature for the suppressing the population of B. brassicae in future.

In temperature regimes study the temperature play an important role in parasitism, development, adult longevity and as well as on adult emergence. The very low temperature and very high temperature affect all these parameters because at high temperature the development increased but adult longevity decreased. Due to decreased in adult longevity the fecundity rate of parasitoid decreased which decreased the emergence as well as percentage parasitism. The suitable temperature for parasitoid parasitism and adult emergence of D. rapae were 21-25 ºC. The relationship of temperature regimes with percentage parasitism and adult emergence was positive but not strongly relate in both cases such, percent parasitism and adult emergence. According to result of this experiment,

temperature predict the presence of D. rapae in B. brassicae colonies when temperature at 21-25 ºC.

Conclucions

The present study showed that development and parasitism rate of D. rapae with B. brassicae as a host was greatly influenced by temperature. Temperature ranging from 20 and 25 °C resulted in a short developmental time and highest percentages of parasitism and emergence. Th temperature range of 20-25 ºC was best for rearing D. rapae on B. brassicae as a host at laboratory conditions. Further studies are needed under field conditions.

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

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The Role of Antioxidants in Sepsis Management: A Review of Therapeutic Applications

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Abstract

Sepsis represents a life-threatening clinical condition characterized by a dysregulated host response to infection leading to organ dysfunction. It affects approximately 49 million people worldwide each year and contributes to an estimated 11 million deaths, accounting for 19.7% of global deaths. Despite the global decline in mortality rates, approximately 25% of patients still succumb to sepsis and hospital mortality in septic shock, a subset of sepsis, approaches 60%. Sepsis not only triggers a multifaceted immune imbalance involving both pro- and anti-inflammatory pathways, but also induces coagulation and complement cascades that collectively contribute to progressive tissue damage and multi-organ dysfunction. While advances have been made in the treatment of sepsis, mortality remains significantly high, ranging from 20% to 80%. The outcome of sepsis can be influenced by numerous factors, including the overall health status of the patient, the severity of sepsis, the organ systems affected and the timing of treatment initiated. Despite the potential of antioxidants such as melatonin, N-Acetylcysteine, vitamin C, vitamin E and selenium to manage oxidative stress in sepsis, further randomized controlled trials are warranted to clarify their dosage, timing and duration of administration and thereby improve our understanding and effective use of these agents in sepsis management. This review aims to examine the relationship between sepsis and antioxidants, specifically focusing on the role of antioxidants in the pathophysiology of sepsis and their potential therapeutic applications.

Keywords: Sepsis, Mortality Rates, Antioxidant Therapy, Septic Shock, Organ Dysfunction.

Introduction

Sepsis is a life-threatening clinical condition characterized by widespread physiological and biochemical disturbances. The Third International Consensus (Sepsis-3) defines sepsis as "organ dysfunction caused by a dysregulated host response to infection" and emphasizes for the first time the vital role of the innate and adaptive immune response in the development of the clinical syndrome (1). Approximately 49 million people are affected by sepsis each year, with an estimated 11 million deaths caused by the syndrome, up to 19.7% of all deaths (2). Globally, although mortality rates seem to be decreasing on

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average, 25% of patients still succumb to sepsis. Septic shock is a subgroup of sepsis characterized by circulatory, cellular and metabolic abnormalities, with a hospital mortality rate approaching 60% (3).

In contrast to an uncomplicated and localized infection, sepsis is a multifaceted disruption of the finely tuned immune balance of inflammation and anti-inflammation. Upregulation of pro- and anti-inflammatory pathways leads to system-wide release of cytokines, mediators and pathogen-related molecules, resulting in activation of coagulation and complement cascades (4). Recognition of pathogen-derived molecular patterns (PAMPs, e.g. endo- and exotoxins, lipids or DNA sequences) or endogenous host-derived danger signals (damage-associated molecular patterns; DAMPs) is the initial signal. These molecules activate specific receptors (toll-like receptors, TLRs) on the surface of antigen-presenting cells (APCs) and monocytes, thus initiating the clinical syndrome of sepsis through transcription of genes involved in inflammation, cell metabolism and adaptive immunity. As both pro-inflammatory and anti-inflammatory pathways are upregulated, the resulting inflammation leads to progressive tissue damage and ultimately causes multi-organ dysfunction. In many patients, concomitant immunosuppression caused by downregulation of activating cell surface molecules, increased apoptosis of immune cells and T cell exhaustion leads to "immunoparalysis" in the later stages of the disease, rendering affected patients susceptible to nosocomial infections, opportunistic pathogens and viral reactivation (5, 6).

This review aims to examine the relationship between sepsis and antioxidants, especially the role of antioxidants in the pathophysiology of sepsis and their potential therapeutic applications. Furthermore, the efficacy of antioxidants in the treatment of sepsis and the current scientific evidence in this field will also be discussed.

Clinical Features: Sepsis is usually characterized by prominent clinical signs such as fever, tachycardia and tachypnea, but these symptoms are far from specific and can also occur with various types of infection and other inflammatory conditions. Especially in elderly patients, these classic signs may be more vague or completely absent. Patients with sepsis often show neurologic signs such as changes in consciousness, confusion and agitation. This may be more pronounced especially in elderly individuals and in patients with immunodeficiency (7) .

Effects on the cardiovascular system are characterized by hypotension and increased heart rate, which means that circulation is compromised and tissues are not adequately perfused. This can lead to organ dysfunction and potentially life-threatening conditions. In particular, renal dysfunction in patients with sepsis can manifest as oliguria or anuria and increased serum creatinine levels. Hepatic dysfunction may be indicated by elevated bilirubin and transaminases, reflecting the involvement of the liver in the inflammatory response and removal of toxins (7, 8). The respiratory system may be severely affected during sepsis, resulting in rapid respiration and hypoxemia. Pulmonary infiltrates may be among the radiologic findings, especially in pulmonary infections such as bacterial pneumonia. Hematologically, sepsis has been associated with coagulopathy and disseminated intravascular coagulation (DIC), which can lead to hemorrhagic complications and microvascular thrombosis (9). There is a wide spectrum of clinical features of sepsis and this may vary depending on factors such as the patient's immune status, age, comorbid conditions, and the agent and site of infection. Therefore, early diagnosis and management of sepsis requires careful evaluation of clinical findings and laboratory data and rapid implementation of appropriate treatment strategies.

Sepsis and Mortality: Influencing Factors and Management Strategies: Despite new developments in the treatment of sepsis, the mortality

rate is high. Mortality rates have been reported to be between 20% and 80%. The different mortality rates reported in these studies are due to the heterogeneity of the study groups. The mortality rate is 45-50% in gram-negative bacterial sepsis, 20-30% in gram-positive bacterial sepsis and 15-30% in anaerobic sepsis. When shock, disseminated intravascular coagulation (DIC), Acute Respiratory Distress Syndrome (ARDS) and other organ failure complications develop, mortality rates range from 70% to 90%. Mortality rates also vary depending on the cause. The highest mortality rate has been reported in Pseudomonas aeruginosa sepsis (10).

The outcomes of sepsis vary depending on a number of factors including the general health status of the patient, severity of sepsis, affected organ systems and how early treatment is initiated. Sepsis may result in severe morbidity and mortality, especially if not recognized and managed early. The prognosis is often serious, especially in patients with septic shock and multiple organ failure. Septic shock can severely affect the patient's cardiovascular system, resulting in low blood pressure, low tissue perfusion and ultimately organ failure (7). Multiple organ failure is a common complication in patients with sepsis and may have serious effects on the kidneys, liver, lungs and other vital organs.

Many studies on sepsis outcomes have shown that early diagnosis and management, especially rapid initiation of appropriate antimicrobial therapy, can significantly improve patient outcomes. However, the management of patients with sepsis is not limited to early antibiotic therapy. Hemodynamic support, strategies to protect and support organ function, and appropriate supportive care are also critical (11).

In terms of long-term outcomes, patients who have survived sepsis may experience physical, cognitive and psychological problems. This condition, known as post-sepsis syndrome, is characterized by decreased quality of life, physical dysfunction and cognitive decline. In addition, patients with sepsis are at high risk for recurrent infections and long-term mortality (12).

Physiopathology: Sepsis is characterized by infectious agents and the systemic inflammatory response syndrome (SIRS) they trigger. The pathophysiology of sepsis is determined by the entry of microorganisms and their toxins into the body, followed by activation of inflammatory and immune responses. This process involves the release of proinflammatory and anti-inflammatory cytokines, coagulation factors and a number of other bioactive molecules (Figure 1).

The body develops a series of complex responses to infectious agents. This response is directed at destroying pathogens, regulating inflammation and repairing tissues. However, in the case of sepsis, this response can be excessive and dysregulated, often leading to harmful and potentially fatal consequences.

Innate immunity serves as the body's first line of defense against pathogens. During sepsis, immune cells such as macrophages and neutrophils phagocytize pathogens and release inflammatory mediators. However, over-activation can lead to systemic inflammation and endothelial damage.

Sepsis also has a significant impact on the coagulation system. Proinflammatory cytokines trigger a cascade that results in endothelial damage and thrombin generation. This can lead to microvascular thrombosis, disseminated intravascular coagulation (DIC) and ultimately organ dysfunction.

During sepsis, the body also engages anti-inflammatory mechanisms. This is intended to stabilize and control the inflammatory response. However, this process can also lead to immunosuppression, making patients more susceptible to secondary infections.

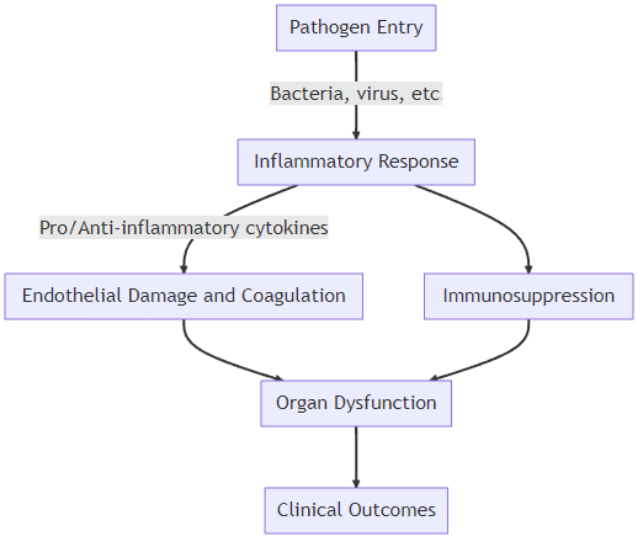

Figure 1. Pathogenesis of sepsis.

The end result of sepsis is a widespread organ failure, commonly known as multiple organ dysfunction syndrome (MODS). Disruptions in inflammatory and coagulation pathways lead to microvascular thrombosis, hypoperfusion and ultimately cellular death (13).

Treatment in Sepsis: Sepsis is a potentially life-threatening condition triggered by a serious infection in the body, leading to organ damage. Treatment of sepsis involves controlling the infection, supporting organ function and improving the general condition of the patient (Figure 2).

1. Control of Infection: Control of infection is critical in the treatment of sepsis and this is usually accomplished through two main strategies: antimicrobial therapy and control of the source of infection. In the context of antimicrobial therapy, early administration of broad-spectrum antibiotics and initial antibiotic selection considering the likely pathogens and local antibiotic resistance patterns are of vital importance (14). After the initial 48-72-hour treatment period, the antibiotic regimen should be re-evaluated according to clinical response and culture results and narrowed if necessary (15). The optimal duration of antibiotic treatment usually varies between 7-10 days, depending on the source of infection, pathogen and clinical response of the patient (16). On the other hand, the infection source control strategy involves surgical removal of infection sources such as infected devices or necrotic tissue (17). Procedures such as drainage of infected fluid collections and gastrointestinal decontamination may also be important in infection control (18). Furthermore, management of infected catheters and other medical devices plays a critical role in controlling infection and preventing secondary infections (19).

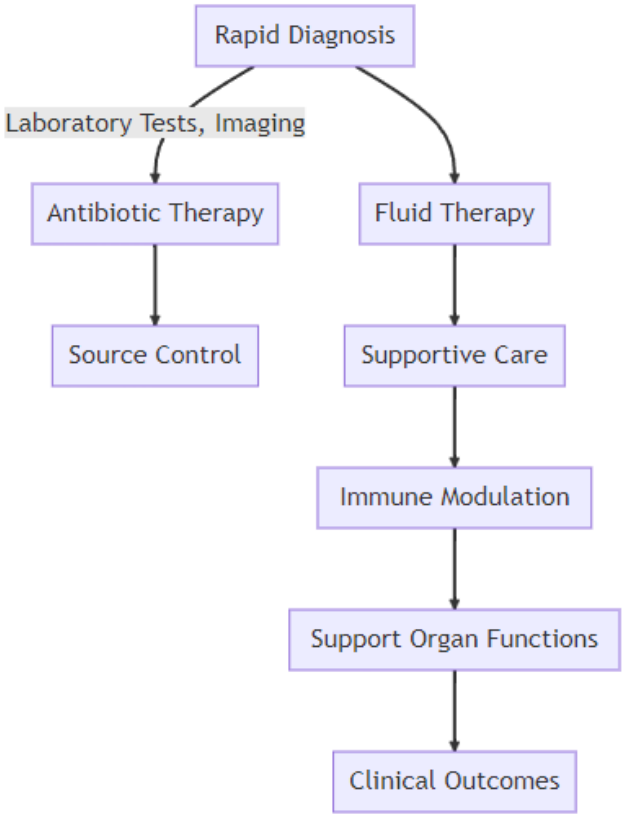

Figure 2. Treatment of Sepsis.

2. Hemodynamic Support and Fluid Management: Sepsis increases vascular permeability, leading to decreased intravascular volume and consequently hypotension, which compromises organ perfusion and can potentially lead to multiple organ dysfunction. Fluid resuscitation is generally considered the first step, which aims to stabilize the patient's hemodynamic status and optimize organ perfusion (20). Fluid therapy should be

guided by dynamic and static hemodynamic monitoring to assess the patient's volume status and perfusion (21). However, fluid management should be cautious because excessive fluid resuscitation may lead to complications, especially pulmonary edema and abdominal hypertension (22). Vasopressor agents, especially noradrenaline, are used in patients who do not respond to fluid resuscitation to maintain adecuate perfusion pressure (23). In addition, inotropic support (e.g. dobutamine) is also required in patients with myocardial dysfunction or characterized by low cardiac output despite fluid resuscitation and vasopressors (11). Hemodynamic support strategies should be individualized and continuously evaluated depending on the patient's clinical condition and response.

3. Organ Support: Respiratory Support: Acute Respiratory Distress Syndrome (ARDS) is a common complication in patients with sepsis, leading to alveolar damage, impaired oxygenation and the need for mechanical ventilation. From a physiologic perspective, using low tidal volumes (6 ml/kg ideal body weight) may reduce ventilator-related lung injury and this approach has reduced mortality in patients with ARDS (24). Positive end-expiratory pressure (PEEP) helps prevent alveolar collapse and improve oxygenation, but there is no consensus on the optimal level of PEEP and this requires titration according to the patient's hemodynamic status and oxygenation response (25).

Renal Support: Sepsis is frequently associated with acute kidney injury (AKI), which can lead to a number of complications including electrolyte imbalance, acidosis and fluid imbalance. Hemodialysis and continuous renal replacement therapies (CRRT) can support renal function and optimize metabolic homeostasis (26). CRRT may be preferred in hemodynamically unstable patients because it provides more stable fluid balance and azotemia control. Hemodialysis may provide faster azotemia control and better fluid output in more stable patients, but there is no clear evidence on the effect of these methods on mortality (27).

4. Metabolic and Nutritional Support: Sepsis leads to a significant increase in energy expenditure and thus catabolism, resulting in protein breakdown, loss of muscle mass and malnutrition. Physiologically, meeting patients' energy and protein requirements is critical to prevent muscle atrophy and promote recovery. Enteral nutrition is generally preferred to preserve gastrointestinal function and prevent bacterial translocation (28). However, optimal energy and protein intake is still a matter of debate in patients with sepsis and may require titration according to the metabolic status and requirements of individual patients (29).

5. Immunomodulation: Sepsis both superactivates and suppresses the immune system, putting the patient at risk for secondary infections and nosocomial infections. Immunomodulation can help the body fight infection more effectively by modulating macrophage activation, cytokine release and T cell function. However, the use of immunomodulatory agents should be cautious, especially regarding which patients will benefit from this treatment and which agents should be used. Several studies have investigated the role of immunomodulation in patients with sepsis, but the data in this area are complex and more research is needed (30).

Role of Antioxidant Therapy in Sepsis: Sepsis leads to the triggering of an excessive inflammatory response in the body and overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species can cause various biological damages associated with oxidative stress, such as lipid peroxidation, protein modification and DNA damage. Oxidative stress has been directly associated with cellular damage, organ dysfunction and death in sepsis (Figure 3) (31).

Antioxidants are components that can neutralize the harmful effects of ROS and RNS and thus reduce oxidative stress. In the context of sepsis, antioxidants can be used to reduce cell damage, modulate the inflammatory response and potentially improve patient prognosis (7, 31, 32). However, the role and efficacy of antioxidants in the treatment of sepsis is still being actively investigated in the current scientific literature.

Mitochondrion-targeted antioxidants (MitoQ, MitoE): Their Potential in Sepsis Management and ROS Mitigation: Given the significant role of the mitochondrial respiratory chain as a primary source of ROS in living cells and the notable involvement of mitochondrial dysfunction in the pathogenesis of sepsis, there's a growing interest in antioxidants targeting the intra-mitochondrial environment. This might be pivotal in mitigating the deleterious effects of sepsis. Many antioxidants are tethered to lipophilic cations like triphenylphosphonium (TPP) to enhance their penetration and concentration in the mitochondria. This is due to the high negative potential inside the inner mitochondrial membrane, which can lead to an increase in their accumulation by 100 to 500-fold (33).

In specific studies, administering mitochondrion-targeted antioxidants like MitoQ or MitoE, and melatonin, to rats infused with LPS/peptidoglycan G, has shown substantial improvements.

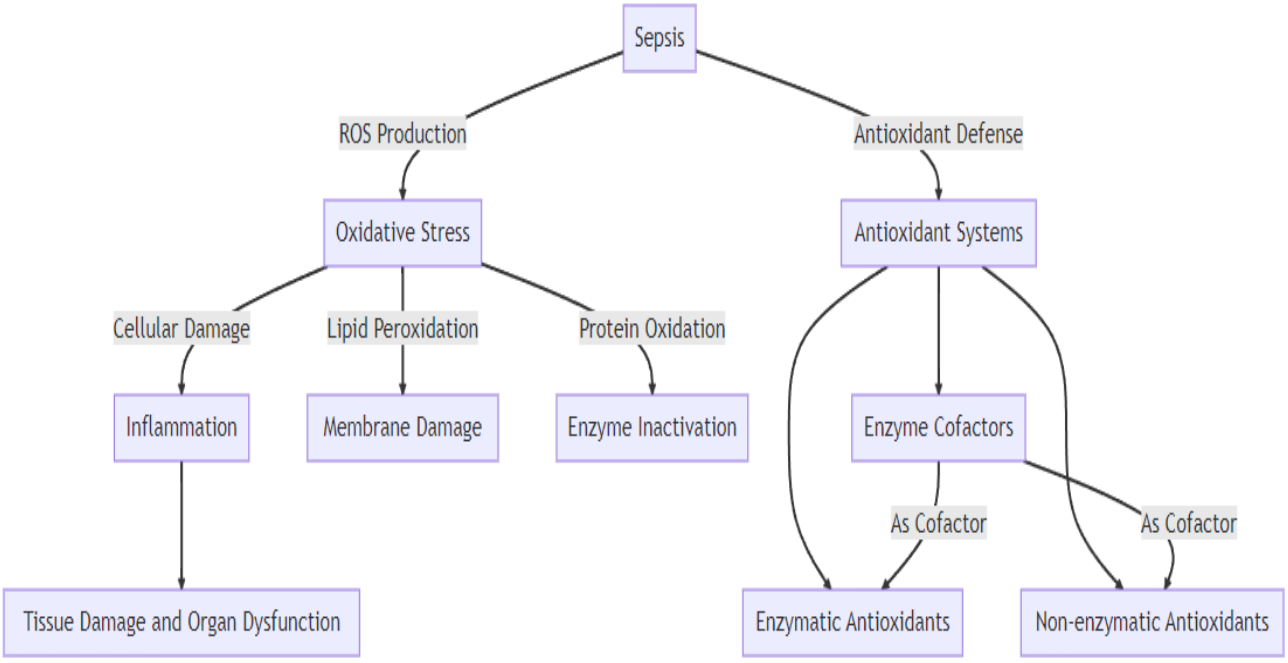

Figure 3. The role of antioxidant systems and enzyme cofactors in the management of sepsis and ROS generation.

These encompass better mitochondrial respiration, reduced oxidative stress, and diminished IL-6 levels due to the infused bacterial cell wall components. Additionally, antioxidant-treated rats demonstrated decreased plasma alanine amino-transferase and creatinine concentrations compared to their untreated counterparts (34). In another experiment, rats injected with LPS and treated with MitoQ exhibited prevention of endotoxin-induced reductions in cardiac mitochondrial function. Notably, this led to ameliorated oxygen consumption by cardiomyocytes, improved contractile functions, and reduced formation of protein carbonyl groups in cardiac tissue (35). Furthermore, long-term administration of MitoQ in mice primarily accumulated in the heart without manifesting symptoms of general toxicity or other

negative metabolic effects. Intriguingly, this long-term administration was associated with a decrease in visceral fat deposits and a reduction in plasma triglycerides (36). In particular, studies on the potential role of antioxidants such as vitamin C, melatonin, N-Acetylcysteine and vitamin E in the management of sepsis and septic shock provide important information in this regard (37-41) .

Melatonin's Protective Role in Sepsis: Oxidative Stress Mitigation and Cellular Restoration: Melatonin is known for its free radical scavenging properties and protects cell membrane lipids, cytosol proteins and nuclear and mitochondrial DNA. Melatonin administration has been observed to significantly reduce lipid peroxidation. The favorable effects of melatonin on sepsis have been associated with inhibition of apoptotic processes and reduction of oxidative stress (38, 39, 42). However, the mechanisms of action of melatonin are not yet fully known and more research is needed in this regard. In a separate research led by Escames et al., the potential therapeutic benefits of melatonin were assessed using an in vivo sepsis model in mice through cecal ligation and puncture (CLP). Findings revealed that sepsis led to an upregulation of iNOS and mtNOS expressions, which coincided with oxidative stress, disturbances in the respiratory chain, and diminished ATP generation, even though ATPase concentrations remained unchanged. Notably, the increase in mtNOS was linked to mitochondrial malfunctions specifically in the cardiac region. Importantly, administering melatonin mitigated the elevated expressions of iNOS/mtNOS triggered by sepsis, conserved mitochondrial balance during septic conditions, and reinstated ATP synthesis (43). In a related experiment involving rats subjected to the CLP method, sepsis led to a decline in GSH concentrations while causing a surge in MDA levels and increased MPO activity, indicating heightened neutrophil clustering. This was observed across multiple tissues including the liver, kidney, heart, lung, diaphragm, and brain. Concurrently, there was a rise in plasma AST, ALT, BUN, and Creatine levels. However, when the rats were treated with melatonin (10mg/kg i.p.) both 30 minutes before and 6 hours post-surgery, notable changes were observed. Specifically, the antioxidant effectively negated the heightened MDA levels across all examined tissues. It restored GSH concentrations to near-normal in the liver, kidney, diaphragm, and brain, although not in the heart and lungs. Furthermore, MPO activity was decreased across all tissues, and there was a significant reduction in elevated AST, ALT, BUN, and Cre values. The researchers posited that melatonin's ability to replenish GSH concentrations and curtail neutrophil accumulation might be behind these observed benefits (44).

N-Acetylcysteine (NAC): A Potential Antioxidant Shield Against Sepsis-Induced Organ Dysfunction and Inflammation: N-acetylcysteine (NAC), another antioxidant, has demonstrated protective qualities in sepsis scenarios. N-Acetylcysteine (NAC) can increase antioxidant capacity and shows a trend towards increasing glutathione (GSH) levels, but this difference is not statistically significant (45). NAC may reduce organ failure, confirming previous findings (46). In a particular study, NAC was given to subjects 20 minutes following the induction of sepsis via LPS injection. Throughout the subsequent 48 hours, researchers closely observed the mean arterial pressure (MAP) and HR. Key biochemical metrics, such as BUN, Cre, LDH, CPK, ALT, AST, TNF-a, IL-6, and IL-10 levels, were assessed. Results indicated that LPS introduction led to marked increases in BUN, Cre, LDH, CPK, ALT, AST, TNF-a, IL-6, and IL-10 levels, alongside HR, while causing a drop in MAP. Intriguingly, NAC administration counteracted some of these changes: it helped moderate the MAP decline, temper the HR surge, and reduce both organ injury indicators (like BUN, Cre, LDH, CPK, ALT, and AST)

and inflammatory markers (including TNF-a, IL-6, and IL-10) attributable to sepsis (47). In a separate study, NAC was administered an hour prior to exposing rats to endotoxin. Observations revealed that NAC reduced lung NF-kB activation in a dose-responsive manner (ranging from 200 to 1000 mg/kg) and curtailed cytokine-induced neutrophil chemoattractant mRNA expression within lung tissues. Consequently, NAC appears to lessen the pulmonary inflammatory response by suppressing NF-kB activity, offering potential relief from certain sepsis-associated respiratory issues (48).

The Roles of Vitamins, Polyphenols, and Other Nutritional Interventions: Common antioxidants, including vitamins C and E, have shown effectiveness in alleviating sepsis effects across multiple organs. The use of vitamin C in sepsis and septic shock is remarkable, especially in relation to its effects on organ dysfunction and mortality. Vitamin E tends to reduce lipid peroxidation (LPO) and carbonylation. This vitamin protects cell membranes from LPO and terminates the chain reaction. It also acts as an O2- and OH scavenger. Other antioxidants, such as polyphenols, β-glucan and antioxidants targeting mitochondria, selenium salts and selenium organ compounds may also be effective in ameliorating oxidative stress in sepsis (49).

In patients with sepsis, initially low vitamin C levels are frequently observed and this has been associated with organ failure and mortality (50). With the administration of vitamin C, restoration of normal vitamin levels and improvement in organ function have been observed. In particular, statistically significant differences were found with Vitamin C administration in patients with pneumonia (51, 52). However, the CITRIS-ALI study showed that Vitamin C administration did not improve in patients with acute respiratory distress syndrome (ARDS) and organ failure (53). These different results may be related to factors such as the timing of treatment initiation. A study involving rats revealed that administering ascorbic acid post-sepsis led to mitigated impacts on several liver functions and molecular markers. Notably, the mRNA expression of certain inflammatory indicators and hepatic enzymes was balanced with ascorbic acid treatment (54). Another study showed that both vitamin C, when given post-sepsis, and vitamin E, when provided before the septic event, stabilized liver functions and certain enzyme activities (55).

In separate research, the protective effect of b-glucan against sepsis-induced oxidative damage in rats was explored. Administered both pre and post a CLP procedure, b-glucan was found to effectively counteract the oxidative stress markers and tissue damages that sepsis typically causes (56).

Regarding nutritional interventions, a study on patients with severe sepsis showcased the potential of an antioxidant-rich liquid diet named ANOM1. Rich in polyphenols and essential vitamins and trace elements, ANOM1 was continuously infused to patients. Findings revealed that this diet not only enhanced vitamin C concentrations but also reduced oxidative stress markers, hinting at its potential to mitigate multi-organ failure in septic shock patients (57).

Roles and Implications of Selenium in Sepsis Management and Treatment Outcomes: Selenium stands as a vital micronutrient, playing a role in the function of over 30 selenoproteins . These proteins offer a range of biological roles, notably including cellular antioxidant defense, the regulation of thyroid hormones, and contributions to both the humoral and cellular branches of immunity. Breaking down serum selenium distribution, selenoprotein P (SePP) holds around 60%, GSH-Px carries about 30%, and albumin contains between 5-10%. The GSH-Px set of proteins is essential for reducing various hydroperoxides and collaborates with vitamin E to combat lipid peroxidation. The family of thioredoxin reductase enzymes aids in the transformation of H2O2

to water. During sepsis-induced endothelial dysfunction, SePP sticks to the endothelial cells, possibly as a protective action against oxidative stress-induced harm (58). Under catabolic conditions, the rate at which selenium is expelled through urine rises. There are observations suggesting that selenoproteins can suppress NF-κB through redox cellular signaling, thus cutting back on ROS/RNS production and the associated cytokine surge. Patients in critical condition due to sepsis often display diminished selenium levels (59) Measurements below 0.7 μmol/L have been linked to increased death rates and organ failures in ICU patients [6]. Kočan et al. [57] suggested that supplemental treatment using a constant 750 μg/24 h dosage of sodium selenite might offer advantages for septic patients grappling with acute lung injury (60). However, there's an ongoing debate over the solitary use of selenium as a therapeutic solution for sepsis. Kong et al. conducted a meta-analysis, revealing that while adding selenium to the primary treatment for severe sepsis or septic shock didn't alter mortality after 28 days, it did correlate with reduced overall mortality(61).

Conclucions

Sepsis remains a major source of morbidity and mortality worldwide. This complex and multifaceted condition leads to an excessive and dysregulated inflammatory response triggered by infection and ultimately organ dysfunction. Management of sepsis involves multiple components including infection control, hemodynamic support, organ support therapies, and appropriate nutritional and metabolic support. The key to sepsis treatment is early diagnosis and rapid intervention, because the earlier treatment is initiated, the more favorable the patient's prognosis.

Antioxidants can potentially play an important role in the management of sepsis, as these agents can reduce oxidative stress, which is prominent in sepsis and leads to cell damage, organ dysfunction and potentially death. Antioxidants such as melatonin, N-Acetylcysteine, vitamin C, vitamin E and selenium have shown promising results in models of sepsis and septic shock, but the clinical efficacy of these agents is still unclear and further research is needed.

Future research should consider factors such as dosage of antioxidants, timing of administration and duration of treatment, and more randomized controlled trials should be conducted to evaluate the clinical efficacy of these agents in sepsis and septic shock. In addition, the long-term outcomes and effects of antioxidants on quality of life in patients with sepsis should also be examined.

These and other novel strategies in the management of sepsis and septic shock may help optimize treatment and improve outcomes in this serious and common condition. However, more high-quality clinical research is needed to evaluate the efficacy and safety of these strategies. This will shape future advances in the management of sepsis and septic shock and provide critical information to help tackle these challenging conditions.

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Acknowledgements

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