

Research Article

Effects of Acetone Extract of Green Tea (*Camellia sinensis*) on Diameter, Viability, and Germinal Vesicle Breakdown Rate of Zebrafish Oocytes (*Danio rerio*) Exposed to Heat Stress as an Animal ModelHabib Syaiful Arif Tuska^{1*}, Aaliyah Nurul Hidayah¹, Bonick Kartini Lonameo¹, Umar Bello², Budiono³

- ¹ Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia
² Ministry of Animal Health and Fisheries Development, Sokoto, Nigeria
³ Gajayana University, Malang, Indonesia
* Email: tuska.hsa@ub.ac.id

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Abstract: Global warming is characterized by increasing temperatures of the earth's atmosphere, ocean and land, resulting in extreme climate change. The effects of climate change will affect animal health through increased ambient temperature (heat stress). This study using Zebrafish as animal model like our previous study because Zebrafish, have 70% identical genetic similarity to humans. In addition, Zebrafish have specific approval from the U.S. Food and Drug Administration for new drug discovery research. Heat stress negatively affects animal health through an increase in Reactive Oxygen Species (ROS), especially in the reproductive system because it causes a decrease in reproductive efficiency, such as follicular development, oocyte quality, and oocyte maturation. Antioxidant supplementation, namely green tea extract, is needed to reduce the negative effects of heat stress. The purpose of this study was to analyze the difference of 4 µl dose of green tea extract (*Camellia sinensis*) on the diameter, viability, and Germinal Vesicle Breakdown (GVBD) rate of Zebrafish oocytes at normal temperature (28°C) and with exposure to heat stress (32°C; 34°C). Zebrafish oocytes were collected and grouped into 2 (two) treatment groups including, non-heat stress group (28°C) and heat stress group (32°C; 34°C) with 6 repetitions. Quantitative data were processed using SPSS software with Two Way ANOVA analysis. The results of Two Way ANOVA showed that green tea extract supplementation had a positive effect on the diameter, viability, and GVBD of Zebrafish oocytes. This is indicated by the significance of the percentage of diameter (P=0.002), viability (P=0.003), and GVBD rate (P=0.000).

Keywords: antioxidants, heat stress, oocytes, ROS, Zebrafish.

INTRODUCTION

Global warming has been an international concern since the industrial revolution. Global warming is characterized by increasing atmospheric, ocean, and land temperatures on earth. The occurrence of global warming causes a series of extreme phenomena in nature in the form of weather and climate changes that can pose a threat to the sustainability of life (Anisa et al., 2021). The effects of weather and climate change will affect animal health through an increase in the temperature, frequency, and intensity of heat waves, known as heat stress (HS) (Lacetera, 2019). Heat stress has a negative impact on the reproductive system through decreased reproductive efficiency, such as decreased oocyte quality, follicle development, and oocyte maturation (Li et al., 2016).

Heat stress can cause oxidative stress through an increase in ROS (reactive oxygen species). The optimal temperature range for Zebrafish is between 24°C and 28°C (Chowdhury & Saikia, 2020). At 32°C, they begin to show signs of thermal sensitivity (Scott & Johnston, 2012), and temperatures of 34°C indicate thermal stress (Residiwati et al., 2024). The animal body has a natural defense system, endogenous antioxidants, to offset the effects of ROS (Guo et al., 2021). However, when the production of these endogenous antioxidants is insufficient and ROS continues to increase, exogenous

antioxidants are needed to prevent prolonged oxidative stress. Exogenous antioxidants are mostly derived from foods, fruits, and plants (Islam et al., 2022).

In order to explore the potential antioxidant of green tea extract in vertebrate organisms, we examined its green tea extract effects in a Zebrafish model under heat stress treatment. Wang et al. (2019) was reported that Zebrafish is a small freshwater teleost that is transparent, fast growing, high fecundity, low cost and exhibits highly conserved developmental programs. Moreover, the housing and husbandry space. In addition Zebrafish have become widely used and are now a globally accepted model in toxicological research, with specific approval from the U.S. Food and Drug Administration for new drug discovery research.

Green tea is a well-known plant of Southeast Asia, including Indonesia, which is known for its medicinal properties, such as antioxidant, anticarcinogenic, antimutagenic, and antihypertensive (Novilla et al., 2022). These properties are attributed to the antioxidant content in green tea, namely polyphenols (catechins), which can work by inhibiting the oxidative chain, thus preventing oxidative stress (Musial et al., 2020). According to our previous study, Residiwati et al. (2024) was reported that orange peel as an antioxidant can enhance the oocyte diameter, survival rate, and GVBD rate of Zebrafish oocytes under non-heat stress and heat stress treatment. Therefore, heat stress treatment was conducted to determine the effectiveness of green tea extract supplementation containing polyphenol antioxidants on the diameter, viability, and germinal vesicle breakdown rate of Zebrafish oocytes.

METHOD

Acetone extraction of Green Tea

Green tea was extracted at the Pharmacy Laboratory, Faculty of Medicine, Islamic University of Malang, which made green tea leaves using the maceration method. Green tea leaves as much as 0.5 kg were steamed for two minutes to dry. Green tea leaves that have been dried are crushed using a cutter or crusher machine until they become powder, then sieved using ASTM (American Standard Test Sieve Series) mesh 14. The sieving results were used as raw material for green tea extraction. The green tea extraction method used 70% acetone solvent in water at a tea and solvent ratio of 1:15 b/v, the extraction process duration was 15 minutes at an operating temperature of 60°C. Acetone was used as an extractant for antioxidant compounds. Mahlo & Eloff (2014) reported that the acetone extract exhibited a remarkable ability to scavenge radicals, strong reducing ability, and was a potential source of natural antioxidants. In addition, Martono et al (2016) was reported that using 60°C for green tea extraction. The results of green tea liquid extract were filtered using 200 - 250 mesh gauze (Shabri & Rohdiana, 2016). In this study, the total polyphenol content in the extract was 60.12%.

Animal Preparation

All protocols were approved by the Institutional Animal Care and Use Committee of Universitas Brawijaya, Malang, Indonesia (No.201-KEP-UB-2023). Zebra fish used in this study were 6-month-old female Zebra fish obtained from the Fish Cultivation Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Zebra fish were acclimatized for 8

days. Then, necropsy was performed on Zebra fish, and oocytes were collected. Oocytes that have been collected were vortexed so that the cumulus complex is separated. Then, washed three times with PBS and observed to take phase 3 (vitellogenesis). After that, it was placed on a 6-well microplate with 2 ml of oocyte culture media. In well numbers 1, 2, 3, 4, and 5, 2 oocytes were placed to observe oocyte diameter, while in well number 6, 20 oocytes were placed to observe oocyte viability and oocyte GVBD rate.

Heat Stress Process

Heat stress exposure was conducted at stage 3 oocytes, or vitellogenesis. Zebrafish oocytes were put into a waterbath with three temperature treatment groups, namely non-heat stress 28°C, heat stress 32°C, and heat stress 34°C.

Supplementation of Green Tea Extract

Supplementation on oocytes in heat stress (HS) or not (NHS) by distinguishing 2 treatment groups, namely the control group (P1) and the extract treatment group (P2). The control group was a group that was not given green tea extract supplementation. However, instead was given a solution (70% acetone) as much as 4 µl as a placebo and 2 ml of culture media in each well. Meanwhile, the extract treatment group was given 4 µl of green tea extract and 2 ml of culture media in each well.

Observation Procedure

The observations of all parameters were made 24 hours after heat stress treatment and supplementation. The diameter of Zebrafish oocytes was observed using microscope with Optilab™ with the help of the ImageJ program. Observation of viability and GVBD rate of Zebrafish oocytes was by staining 0.1% trypan blue as much as 0.4 ml for 40 seconds. After staining, wash three times using distilled water.

In addition, the oocytes were placed on a concave glass object to observe their viability and GVBD rate. Viability and Germinal Vesicle Breakdown (GVBD) Rate were calculated by calculating the total number of oocytes that experience GVBD and dividing it by the total number of oocyte samples, then multiplying by 100%.

Statistical Analysis

All the data parameter including the diameter of the oocyte, viability, and GVBD rate were analyzed using quantitative analysis by SPSS software application. The GVBD rate data that has been obtained is normally distributed and homogeneous ($P > 0.05$) and data analysis is carried out using ANOVA analysis of variance and continued with the LSD post hoc test. Meanwhile, the results of diameter and viability obtained data were not normally distributed and homogeneous, so using the Kruskal Wallis non-parametric test, to determine whether there were significant differences between treatments and continued with the Mann Whitney post hoc test to determine whether there were significant differences between groups.

RESULT AND DISCUSSION

Effects of Green Tea Extract Supplementation on Zebrafish Oocyte Diameter

Oocyte diameter is an important indicator to determine whether the oocyte will acquire further

developmental competence or not (Kawano et al., 2022). Oocyte diameter was calculated by measuring the average length of the diameter from one end of the oocyte to the other end that passes perpendicularly through the center of the oocyte (Figure 1) (Pors et al., 2022).

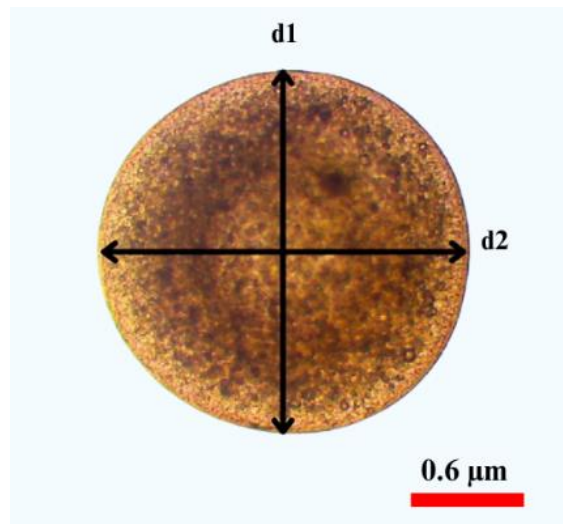


Figure 1. Zebrafish oocyte diameter measurement (d1: diameter 1; d2: diameter 2).

Oocytes will experience a significant increase in size in accordance with the development of the oogenesis phase. In Zebrafish, the size of the oocyte diameter in phase 4 (four) or maturation is around 690-730 μm (Elkouby & Mullins, 2017). The average results of oocyte diameter can be seen in Figure 2.

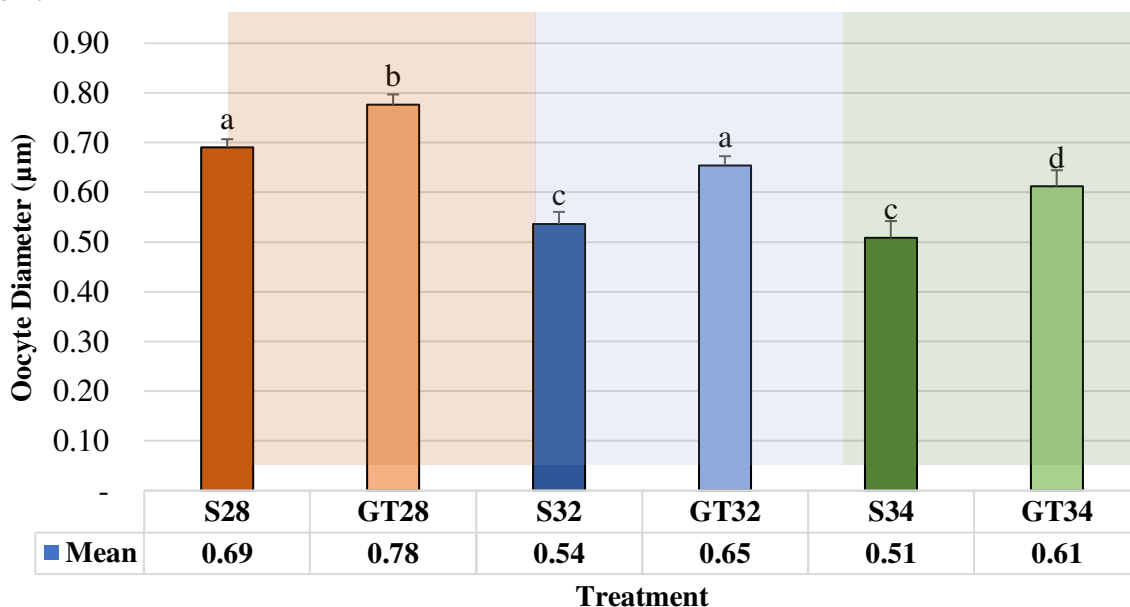


Figure 2. Zebrafish oocyte diameter (GT28: green tea at 28°C, S28: solution temperature 28°C, GT32: green tea temperature 32°C, S32: solution temperature 32°C, GT34: green tea temperature 34°C, S34: solution temperature 34°C; different notations indicate significant differences between treatments).

Based on Figure 2, the results of the Zebrafish oocyte diameter test showed that at normal temperature (28°C), the green tea extract treatment group (GT28) (0.78 ± 0.01 ; mean \pm standard error) was significantly different from the control treatment group S28 (0.69 ± 0.01). In the administration of green tea extract (GT28), the oocyte diameter was 0.09 mm higher than the control group (S28). This indicates that green tea extract at normal temperature has the potential to increase the diameter of Zebrafish oocytes compared to the control group (S28).

According to Teletchea et al. (2009), one of the factors affecting oocyte diameter is the environment, such as optimal temperature can increase oocyte development and diameter along with the phase of oogenesis. Oocytes will mature faster at a larger diameter, because the oocytes have reached the stage of meiosis. Oocyte development is characterized by an increase in oocyte diameter followed by growth in the size of cell organelles, such as golgi bodies, endoplasmic reticulum, lipids, and increased protein synthesis (Parera & Hadisutanto, 2014). Optimal temperature followed by the administration of green tea extract can increase antioxidant status, so as to maintain and even increase the development of oocyte diameter. The GT32 treatment group (0.65 ± 0.01) showed a significant difference with the S32 treatment group (0.54 ± 0.01), and the oocyte diameter of the green tea extract group (GT32) was 0.19 mm higher than that of the control group (S32). The TH34 treatment group at 34 °C (0.61 ± 0.01) showed a significant difference with the S34 treatment group (0.51 ± 0.01), and the oocyte diameter of the green tea extract group (GT34) was 0.1 mm higher than that of the control group (S32).

Kawano et al. (2022) reported that oocytes in heat stress treatment with a temperature of 38.5 °C have a smaller diameter than the non-heat stress treatment of 28 °C. This is because high temperatures can interfere with the production of granulosa cells and theca cells, and interfere with follicular growth, resulting in a decrease in oocyte diameter. Heat stress adversely affects oocyte quality through oocyte shrinkage, follicle depletion, follicle growth inhibition, which can reduce the percentage of oocyte diameter development.

In addition, there is a decrease in estradiol production by granulosa cells and andostenedione production by oocyte teka cells. Heat stress can disrupt the organization of the oocyte cytoskeleton, a network of protein filaments responsible for maintaining its shape and internal structure. This disorganization can lead to a decrease in oocyte diameter and impair the oocyte's ability to undergo the complex rearrangements required for later stages of oogenesis (Wolfenson & Roth, 2019).

In this study, the diameter of oocytes at 32 °C and 34 °C with green tea extract or without green tea extract decreased with increasing temperature, but with green tea extract, the decrease in oocyte diameter was not as low as in the group without green tea extract. This is in line with the research of Zhang et al. (2020), which states that to prevent oxidative stress, antioxidant supplementation is needed in oocyte IVM media, one of which is green tea extract, which can reduce the negative effects of oocyte diameter development by HS exposure. Green tea extract works by inhibiting ROS formation activity and increasing antioxidant enzymes (SOD, catalase, GSH).

According to Mora et al., (2024), green tea extract is able to protect oocytes from heat exposure by increasing oocyte developmental competence and increasing oocyte diameter. According to

(Maleki et al., 2021), green tea extract can reduce the adverse effects of hormonal disorders on the ovaries by reducing gonadotropin secretion and increasing the production of follicular cells and granulosa cells by stimulating the activity of FSH, LH, and estradiol hormones, which are important hormones in follicular development. Granulosa cells surrounding the oocyte will proliferate, so the diameter of the oocyte will increase. Meanwhile, follicular cells and follicular fluid contain nutrients and hormones that oocytes need to grow and develop. If the number of follicular cells increases, the diameter of the oocyte will also increase, indicating that the oocyte has better quality (Da Broi et al., 2018).

In the green tea extract group, the measured oocyte diameter still did not reach the normal standard, although there was a significant difference with the control group. This could be attributed to factors affecting the effectiveness of the antioxidant itself. The effectiveness of antioxidants depends on the concentration, dose, and origin of oxidative stress. In addition, due to the increased temperature accompanied by inappropriate concentration or dose, the antioxidants were not able to reduce ROS, and oxidative stress continued, resulting in the oocyte diameter not reaching the normal standard (Pizzino et al., 2017).

Effect of Green Tea Extract Supplementation on Zebrafish Oocyte Viability

Assessment of oocyte viability is carried out to estimate the damage caused to oocytes through the percentage of living oocytes (Kamiloglu et al., 2020). According to Karimah (2021), the normal standard of oocyte viability is in the range of 80-95%. The stain used is 0.1% trypan blue, dead oocytes will appear colored, while live oocytes appear colorless (transparent) (Figure 3).

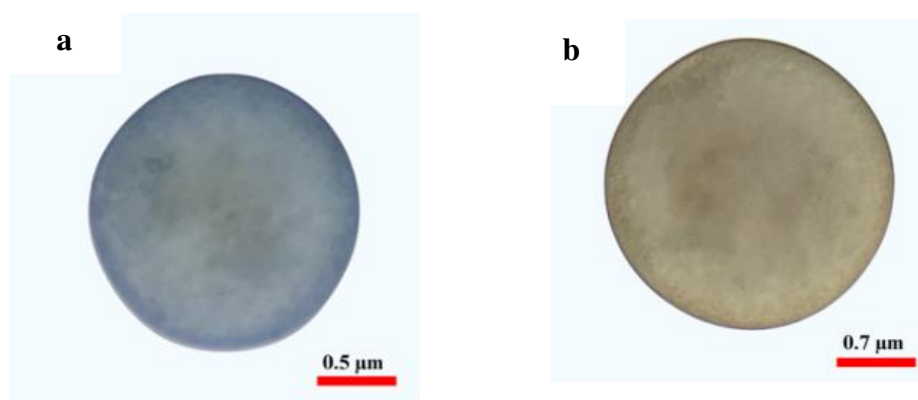


Figure 3. (a) Dead oocytes stained with TB ; (b)live oocytes not stained with TB

Exposure to heat stress can induce oxidative stress, damage the mitochondrial respiratory chain, and alter membrane permeability, leading to mitochondrial dysfunction through increased Reactive Oxygen Species (ROS). Mitochondrial dysfunction leads to reduced cellular adenosine 5'-triphosphate (ATP) which will affect viability. This is because oocyte viability is highly dependent on ATP and nutrient reserves in the mitochondria (Maleki et al., 2021). The average results of oocyte viability can be seen in Figure 4.

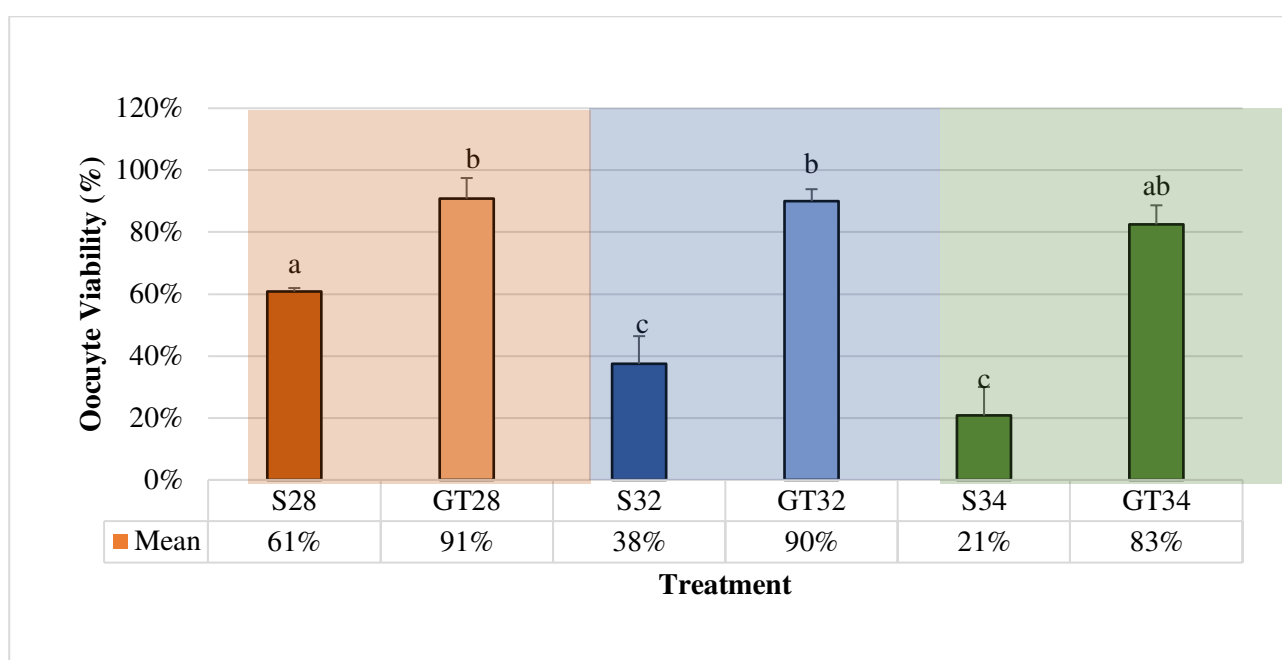


Figure 4. Zebrafish oocyte viability test graph (GT28: green tea at 28°C, S28: solution temperature 28°C, GT32: green tea temperature 32°C, S32: solution temperature 32°C, GT34: green tea temperature 34°C, S34: solution temperature 34°C; different notations indicate significant differences between treatments).

Based on Figure 4, the results obtained at normal temperature (28°C), the green tea extract treatment group (GT28) (91% ± 6%; mean ± standard error) showed a significant difference with the S28 treatment group (61% ± 6%). The normal temperature for Zebrafish oocytes is around 26-28°C. At this temperature, Zebrafish oocytes can survive and mature well in media culture.

The GT32 treatment group at 32 °C (90% ± 5%) showed a significant difference with the S32 treatment group (38% ± 5%) and the green tea extract treatment had a higher viability of 52% compared to the control group (S32). The GT34 treatment group at 34 °C (83% ± 5%) showed a significant difference with the S34 treatment group (21% ± 5%) with an increase in viability in the green tea extract treatment by 62% from the control group.

According to Sakatani (2017), which reported that the group of oocytes given heat exposure at 40 °C showed lower viability compared to oocytes that were not given heat exposure. High temperatures cause a decrease in oocyte viability of about 30-65% (Miller, 2013). During heat exposure, the percentage of saturated fatty acids in oocytes increases, which is too high saturated fatty acids can interfere with the metabolism and transportation of nutrients in oocytes, thus inhibiting oocyte survival. Heat exposure can also affect the biochemical properties or permeability of the oocyte membrane through an increase in ROS, so that the oocyte membrane is susceptible to external damage which causes a decrease in oocyte survival (Berling et al., 2022).

In this study, oocyte viability at 32 °C and 34 °C with green tea extract or without green tea extract decreased with increasing temperature, but with green tea extract, the decrease in oocyte viability was not as low as in the group without green tea extract. This is in line with the research of

Mora et al. (2024), that green tea extract has a positive impact on the quality of oocytes exposed to heat stress due to its antioxidant activity, such as maintaining oocyte viability. In addition, (Reed & Jennings, 2011) reported that temperatures below 25°C and above 30°C reduce the breeding capability of the fish and thus the numbers of embryos produced

Green tea extract contains polyphenol antioxidants that work by counteracting free radicals and regulating the activity of various types of oxidation in the body. This is because the phenolic hydroxyl structure in green tea has a conjugation effect (hydrogen ion binding ability). Therefore, the addition of green tea extract can bind hydrogen ions, thereby neutralizing free radicals and Reactive Oxygen Species (ROS) to form more stable substances (Yan et al., 2020). In addition, under heat stress conditions, there is an increase in HSP70 which protects cells from thermal or oxidative stress and is necessary for cell repair and survival under stress conditions. HSP70 works together with antioxidants, thus maintaining oocyte viability (Belenichev et al., 2023).

Effects of Green Tea Extract Supplementation on Germinal Vesicle Breakdown (GVBD) of Zebrafish Oocytes

Germinal Vesicle Breakdown (GVBD) is a marker of oocyte maturation. Germinal Vesicle Breakdown (GVBD) is a morphological change in the oocyte nucleus that is clearly visible after the meiosis stage restarts, characterized by the cytoplasm becoming clear, larger in size, and the rupture of the GV so that the nucleus will shift to the edge (Kotani et al., 2017; Nur et al., 2017). Illustration of the development of Zebrafish oocytes that experience GVBD can be seen in Figure 5.

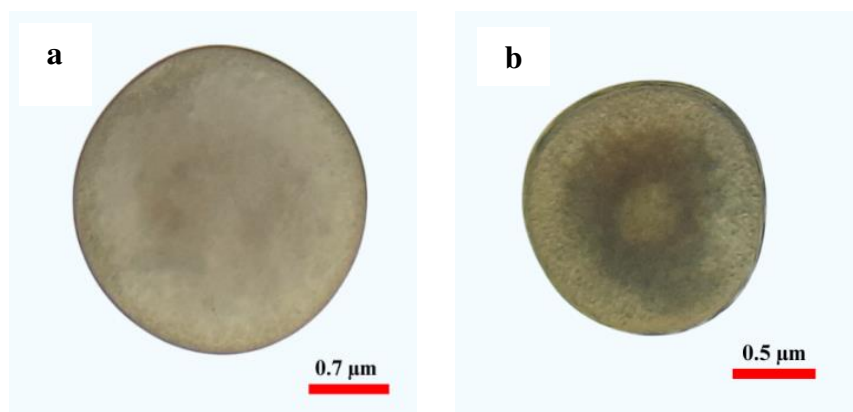


Figure 5. Zebrafish oocyte development; (a) GVBD; (b) GV

The standard percentage of good oocyte GVBD is in the range of 72-87%. The results of the statistical analysis test of Zebrafish oocyte GVBD can be seen in Figure 6.

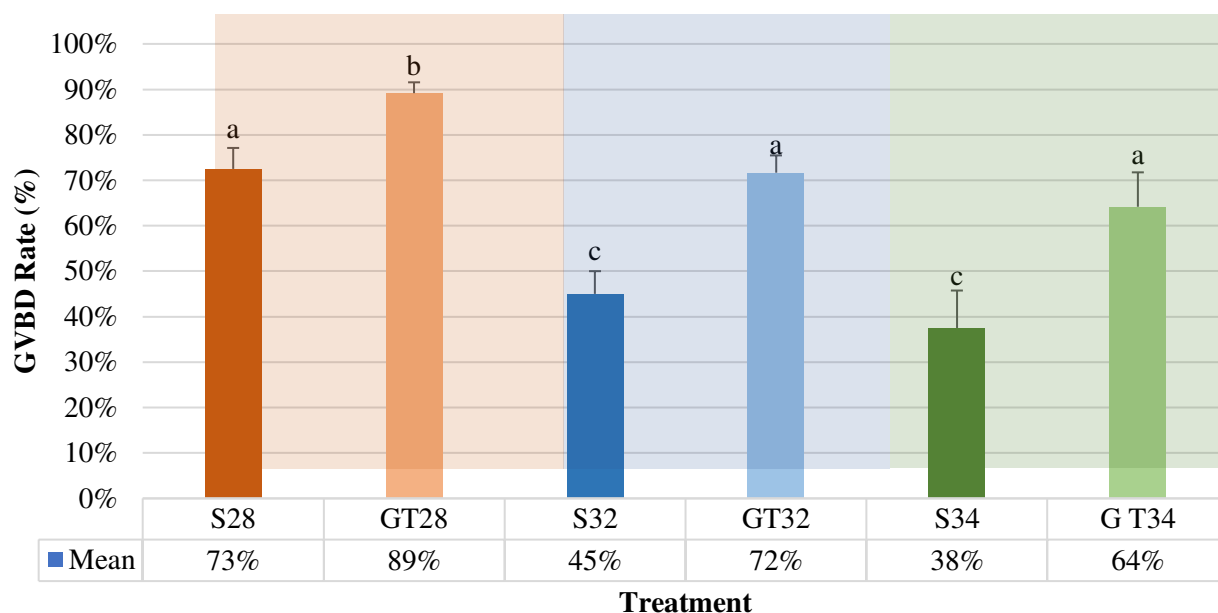


Figure 6. GVBD test graph of Zebrafish oocytes (GT28: green tea at 28°C, S28: solution temperature 28°C, GT32: green tea temperature 32°C, S32: solution temperature 32°C, GT34: green tea temperature 34°C, S34: solution temperature 34°C; different notations indicate significant differences between treatments)

Based on Figure 6, the results of the oocyte GVBD test showed that at normal temperature (28 °C), the green tea extract treatment group (GT28) (89% ± 4%; standard error) is significantly different from the control treatment group S28 (73% ± 4%). This showed that green tea extract has the ability to increase the GVBD of Zebrafish oocytes at normal temperature (GT28) with the control group (S32). In line with the research of Aleström et al., (2020), which states that the optimal temperature for oocyte and embryo development ranges from 24-29 °C. Oocytes that are not exposed to heat stress or are at normal temperature and normal environment, do not experience a decrease in oocyte quality. In line with the research of Mora et al. (2023), at the optimal oocyte development temperature, followed by the administration of green tea extract can increase antioxidant status, so that it will help oocyte maturation (Mora et al., 2023).

The GT32 treatment group at 32 °C (72% ± 4%) was showed a significant difference with the control group S32 (45% ± 4%) and the administration of green tea extract increased 27% from the control group. At 34°C. The GT34 treatment group at 34 °C (64% ± 4%) was showed a significant difference with the S34 treatment group (38% ± 4%) and the administration of green tea extract increased by 26% compared to the control group. We hypothesize that this is due to antioxidants in green tea, especially polyphenols, which can work more optimally to counteract ROS caused by heat exposure at 32°C and 34°C.

This is in line with the research of Kawano et al. (2022), which reported that exposure to heat stress will reduce the competence of oocyte development at the GV stage to GVBD. Heat stress conditions can increase ROS production which causes disruption of oocyte development (Mihalas et

al., 2017). In oocytes that mature normally, the GV will rupture and the chromosomes will be evenly distributed in the cell. However, in oocytes exposed to HS, the GVs may not rupture at all or only partially rupture. Chromosomes may also appear concentrated in one part of the cell (Da Broi et al., 2018). Heat exposure can cause chromosome condensation disorders. Oocytes that have non-condensed chromosomes have a higher risk of oocyte GVBD failure because it inhibits the release of the oocyte nucleus from the cytoplasm. In addition, uncondensed chromosomes have a more fragile structure, so the DNA is not protected by chromatin proteins (Maeshima et al., 2021).

Heat exposure can cause chromosome condensation disorders. Oocytes that have uncondensed chromosomes have a higher risk of oocyte GVBD failure because it inhibits the release of the oocyte nucleus from the cytoplasm. In addition, chromosomes have a more fragile structure, so DNA is not protected by chromatin proteins (Maeshima et al., 2021).

In this study, the percentage of oocyte GVBD at 32 °C and 34 °C with or without green tea extract decreased with increasing temperature, but in the administration of green tea extract, the decrease in the percentage of oocyte GVBD was not as low as in the group without green tea extract. This is in line with Mihalas et al. (2017), which states that the provision of antioxidants in heat exposure can reduce excess ROS production, so as to protect oocytes and potentially facilitate the development of normal GVBD. According to Mora et al. (2023), antioxidants in green tea extract can reduce the adverse effects on oocytes exposed to heat stress by increasing antioxidant enzymes (SOD, GSH, catalase) in oocytes.

According to Chatterjee et al. (2020), green tea extract contains polyphenolic compounds, which have antioxidant properties. These antioxidant properties can protect chromosomes from damage and help increase levels of estrogen hormones, which play a role in chromosome condensation, so as to increase the development of oocyte GVBD. In addition, green tea extract also increases the activity of protein kinase C, a protein that regulates the process of oocyte mitosis and meiosis, so it can increase or accelerate the process of oocyte GVBD. Zhao et al. (2021) was reported that green tea extract can increase the production of growth factors EGF (epidermal growth factor) and IGF-1 (insulin-like growth factor 1) in mouse oocytes, which helps increase the percentage of oocyte GVBD development. In addition, Xie et al. (2016) was reported that IGF-I is another important growth factor which is broadly involved in the folliculogenesis, particularly in the proliferation of granulosa cells. Similar to EGF family, the existing evidence indicates that IGF-I has also been involved in the oocyte maturation in both mammals and fish.

Effects of Green Tea Extract Supplementation on Zebrafish Oocyte Quality

The quality of oocytes in Zebrafish can be influenced by several factors, including environmental factors. Environmental factors can include water temperature, pH, which can affect oocyte development and survival (Gallo et al., 2022). Zebrafish oocyte quality can be assessed using various indicators, including oocyte viability, diameter, and GVBD rate. Oocyte diameter is related to viability and the GV breakdown process (GVBD). Larger oocytes are associated with a greater likelihood of successful in vitro maturation (Khalili et al., 2013).

Large diameter oocytes tend to store more nutrient reserves, such as lipids, proteins, polysaccharides, thus allowing the oocyte to remain viable. Dead oocytes are unable to complete oocyte maturation (GVBD) and the normal development process, this is because the nutritional status of oocytes can affect oocyte quality and subsequent developmental competence (Czerneková et al., 2018). The morphology of good Zebrafish oocytes will appear clear, round or oval in shape, some oocytes are irregular. Meanwhile, damaged oocytes will appear dark, shrunken, or even flat (Kobayashi et al., 2021). The percentage of oocyte quality in this study is shown in Figure 7.

Zebrafish Oocyte Quality

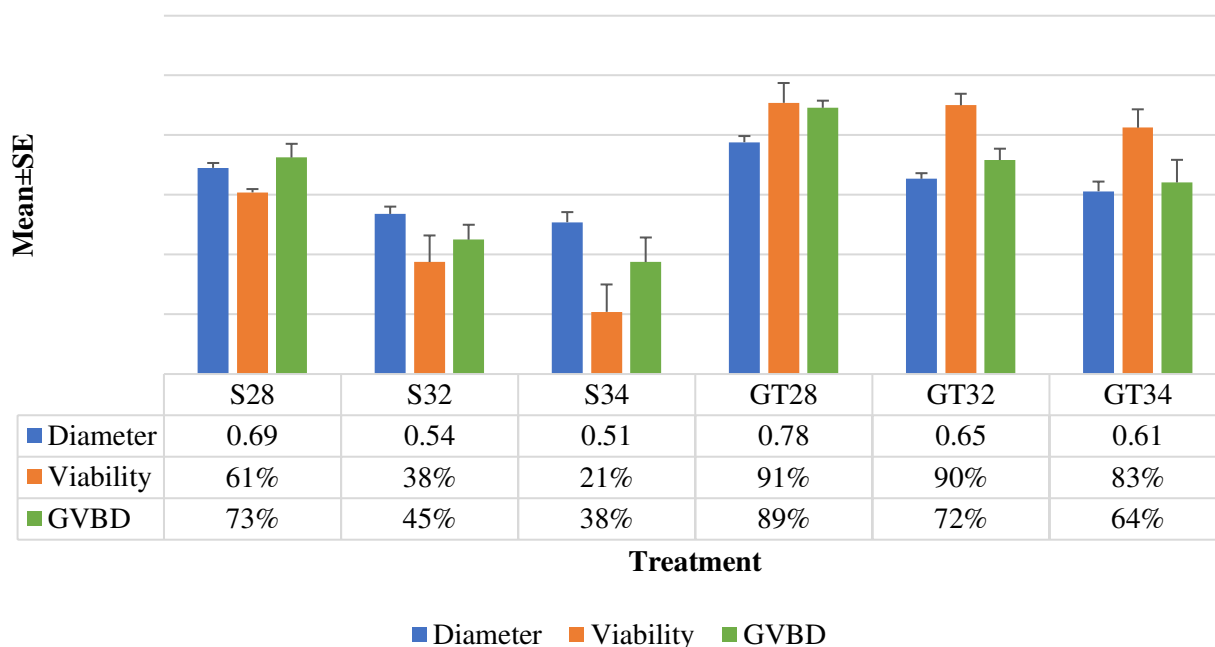


Figure 7. Zebrafish oocyte quality (S28: solution temperature 28°C, GT28: green tea temperature 28°C, S32: solution temperature 32°C, GT32: green tea temperature 32°C, S34: solution temperature 34°C, GT34: green tea temperature 34°C)

Based on Figure 7, the trend seen in the percentage graph of solution treatment or control (S28, S32, S34), that is, there is a decrease in diameter, viability, and GVBD rate parameters, as heat exposure increases (32°C, 34°C). We hypothesize that high temperature affects oocyte development and quality. According to Rakha et al. (2022), a decrease in diameter, viability, and GVBD rate indicates poorer oocyte quality which may be caused by oxidative stress due to heat exposure. Oxidative stress can reduce oocyte development and maturity, as well as oocyte survival rate.

Heat stress induces the release of cortisol (stress hormone), which can inhibit estradiol biosynthesis from follicular cells leading to a decrease in oocyte quality. Oocytes subjected to oxidative stress can experience shrinkage, membrane clumping, granulation, and cytoplasmic

degeneration. In oocytes, granulosa cells play an important role in oocyte development and maturation, as they provide nutrients and paracrine signals necessary for oocyte maturation. However, under HS conditions, there is a decrease in communication between granulosa cells and the oocyte itself, thus affecting the supply of nutrients and oocyte maturation factors that affect oocyte quality (Prasad et al., 2016).

The trend seen in the percentage graph of green tea extract treatment (GT28, GT32, GT34) is that there is a decrease in diameter, viability, and GVBD rate parameters as heat exposure increases (32°C, 34°C). In addition, Reed and Jennings (2011) was reported that above 31°C and below 25°C, Zebrafish probably will not breed and development will be abnormal.

However, when compared to the solution treatment (control), the percentage of oocyte quality in the administration of green tea extract is better. This is indicated by the higher percentage of diameter, viability, and GVBD rate compared to the solution group, followed by a significant difference between green tea extract treatment and solution treatment, which was marked $P > 0.05$ in the follow-up test (LSD, Mann Whitney). We hypothesize that high temperature affects oocyte development and quality with a decrease in oocyte quality, but the administration of green tea extract antioxidants can help improve oocyte quality.

According to (Tripathi et al., 2023), the balance between ROS and antioxidant levels in oocytes is important to maintain good oocyte quality. At normal physiological levels, ROS modulate oocyte function, but accumulation of ROS beyond physiological levels can cause oxidative stress that can reduce oocyte quality.

Therefore, the addition of antioxidant supplements is needed to regulate the level of ROS and improve mitochondrial function, so that the decline in oocyte quality can be prevented. Green tea extract functions as an antioxidant mainly due to its high polyphenol content, especially catechins, namely epigallocatechin gallate (EGCG). These polyphenols can neutralize free radicals, reduce oxidative stress, and protect against cell damage caused by reactive oxygen species. Green tea polyphenols act as direct antioxidants by capturing reactive oxygen species and can also upregulate phase II antioxidant enzymes, thus providing a protective effect on oocytes (Forester & Lambert, 2011).

CONCLUSION

Supplementation of green tea extract has a positive effect on the diameter, viability, and GVBD Rate of Zebrafish oocytes (*Danio rerio*) in both non heat Stress and heat Stress treatments characterized by an increase in oocyte diameter, while oocytes in the heat stress group without green tea extract supplementation decreased in diameter, viability, and GVBD rate.

SUGESSTIONS

Further study needs to observe the quality of oocytes with varying temperatures and doses to better determine the effectiveness of green tea extract in counteracting ROS and restoring the quality of oocytes after exposure to heat stress. It is necessary to add a negative control to determine whether

the solution (70% acetone) has an effect or not on the quality of oocytes in non heat stress and heat stress treatment. Further studies are needed regarding the administration of green tea extract in vivo to find out more details about the effectiveness, toxicity, and activities of green tea extract on the body.

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