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EFFECT OF BLACK GRAPE EXTRACT ON CD4$^+$ and CD8$^+$ EXPRESSION IN MICE INFECTED WITH Salmonella typhimurium

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Abstrak

Salmonelosis adalah penyakit yang disebabkan bakteri Salmonella sp, ditularkan melalui makanan dan minuman yang menyebabkan penurunan ekspresi CD4$^+$. Ekstrak buah anggur hitam memiliki kandungan polifenol, vitamin C, prosianidin, quercetin, resveratrol, antosianidin, flavonoid yang dapat meningkatkan sistem imunitas melalui proliferasi limfosit T CD4+/CD8+. Tujuan penelitian ini adalah untuk mengevaluasi aktivitas ekstrak anggur hitam terhadap ekspresi CD4$^+$ dan CD8$^+$ pada mencit balb/C terinfeksi Salmonella typhimurium. Metode penelitian ini adalah pembuatan ekstrak etanol 96% buah anggur hitam, infeksi bakteri Salmonella typhimurium 10^8 pada mencit, evaluasi bakterimia menggunakan pewarnaan giemsa, terapi ekstrak buah anggur hitam, pemeriksaan flowcytometeri, serta uji statistika menggunakan One Way Anova dengan uji lanjtuan tuckey 95%. Hasil analisa statistika One Way Anova menunjukkan bahwa ekspresi sel T CD4$^+$ (0,72>0,05) dan sel T CD8$^+$ (1,84>0,05) tidak terjadi perbedaan yang nyata. Namun secara biologis, terjadi peningkatan ekspresi CD4$^+$ dan CD8$^+$ pada dosis 100 mg/KgBB jika dibandingkan kelompok lain. Kemudian, terjadi penurunan ekspresi CD4$^+$ dan CD8$^+$ pada dosis 200 mg/KgBB dan 400 mg/KgBB jika dibandingkan dengan dosis 100 mg/KgBB. Kesimpulannya, secara biologis, ekstrak buah anggur hitam dapat meningkatkan ekspresi CD4$^+$ dan CD8$^+$ pada dosis rendah, serta dapat menurunkan ekspresi CD4$^+$ dan CD8$^+$ pada dosis sedang sampai tinggi.

***Kata Kunci**:* Anggur; *Salmonella typhimurium*; CD4$^+$; CD8$^+$

Abstract

Salmonelosis is a disease caused by the Salmonella sp. that causes a decrease in CD4$^+$ expression. Black grape can boost the immune system through CD4$^+$/CD8$^+$ proliferation. The purpose of this study was to evaluate the activity of black grape extract to CD4$^+$ and CD8$^+$ expression in mice infected with *Salmonella typhimurium*. Research method is extract of black grapes, *Salmonella typhimurium* 10^8 infection, bacterial evaluation, extract therapy, flowcytometery examination, and 95% Anova test. The results of Anova test showed that the expression of CD4$^+$ and CD8$^+$ is not different. Biologically, an increase in CD4$^+$ and CD8$^+$ expression at doses of 100 mg/KgBB. A decrease in CD4$^+$ and CD8$^+$ expression at doses of 200 mg/KgBB and 400 mg/KgBB. Conclusion, biologically, black grape extract can increase the expression of CD4$^+$ and CD8$^+$ at low doses, as well as may decrease the expression of CD4$^+$ and CD8$^+$ at moderate to high doses.

***Keywords**: Black grape; Salmonella typhimurium; CD4$^+$ and CD8$^+$ expression*

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1. INTRODUCTION

Salmonelosis is a zoonotic disease (Elbediwi *et al.* , 2021) from Salmonella bacterial infections derived from food and beverages (*food borne disease*). Salmonella bacteria are bacteria that are rod-shaped, gram-negative, and have flagella used for bacterial motility (Mahari and Gandhi, 2022). Salmonella bacteria also belong to the family Enterobacteriaceae (Jassim and Obead, 2022).

Based on data from the *World Health Organization*, there are 11-20 million cases of people in the world who experience typhoid fever every year, and 150,000 people die from the infection (Jahan *et al.* , 2022). Salmonelosis causes gastrointestinal diseases, stomach cramps, bloody diarrhea, fever, myalgia, nausea and vomiting (Ehuwa, Jaiswal and Jaiswal, 2021), in addition, based on immunological studies of Salmonella bacterial infection can inhibit the presentation of dendritic cells, and suppress the migration of dendritic cells, so that the activation process of CD4+ T cells is inhibited (Yadav, 2020).

Based on antibiotic studies, Salmonella is resistant to several antibiotics, namely amoxicillin, tetracycline, cettazidine, chloramphenicol, calistin, and ciprofloxacin (Talukder *et al.* , 2021) . Such resistance causes world health problems, both those originating in developed countries, and developing countries (Wójcicki *et al.* , 2021) . In vivo studies to determine the pathogenicity of Salmonella typhi bacteria in general using mice infected with Salmonella *typhimurium* (Salmonella serovar enterica). *Salmonella typhi* has an 80% genome sequence with *Salmonella typhimurium* but the pathogesity is different (Jahan *et al.* , 2022).

Black grapes (*Vitis vinifera*) contain polyphenols, anthocyanins, flavonols, stilbenes, phenolic acids, proteins, fats, vitamin C, prosianidins, gallic acids, epicatechins, catechins, quercetin, flavonol glycosides, resveratrol, anthocyanidins, caffeine acids, koumaric acid, koutaric acid, ferulic acid, ferturic acid, routine, Quercetin-3-β-D-glucoside, quercitrin, mirisetin, catechins, epicatesin (Insanu *et al.* , 2021) , flavonoids and protoantosianidins (Siswanto *et al.*, 2020) as anztibacterial and immunomodulator.

The immunomodulatory effect of black grape seeds combined with green extract in a ratio of 1:2 can increase CD4+ and CD8+ lymphocyte immunity cells (El-Desouky, Hanafi and Abbas, 2017). Resveratrol can also increase NK cells and simultaneously induce CD4+ and CD8+ lymphocyte cells (Malaguarnera, 2019). Invitropolyphenols can inhibit the production of pro-inflammatory cytokines, namely IL-1, IL-6, and TNF-α induced by LPS (Zhang, 2018). Decreased pro-inflammatory cytokines can prevent excessive tissue damage.

Based on the study above, there is a need for alternative / herbal therapy against Salmonella bacterial infection that can prevent antibiotic resistance and increase immunity, especially the adative immune system, so that it can maximally reduce Salmonella bacterial infection, one of which is by using black grapes (*Vitis vinifera*). The purpose of this study was to evaluate the activity of black grape extract against CD4+ and $CD8^+$ expression in balb/C mice infected with Salmonella typhimurium.

2. RESEARCH METHOD

Some of the stages of the research method include:

2.1 Black Grape fruit extraction

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Proses extraction begins with providing black grape material as much as 5 Kg, then the covenant process is carried out with a temperature of 40ºC for 3 days until it becomes powder. The finished powder is 500 grams, then the extraction process is carried out by the maceration method 1 time using 96% ethanol with a total volume of 4500 ml, with an evaporation time of 41 hours 15 minutes. Then the extract is put in a dark container to prevent direct exposure to the sun, so that the content in the extract remains stable. Then the extract is tested phytochemically to determine the content of flavonoids, saponins, and tannins from the extract.

2.2 Grouping

The mice used were 30 balb/c strains weighing 20-25 grams, then acclimatized in the experimental veterinary laboratory of the Faculty of Health Sciences, Dr. Soebandi University for 7-14 days. After acclimatization, mice were divided into 6 groups namely Negative control (Tanpa infection/normal), positive control (infection + CMC Na), Standard control (S. typhimurium infection + chloramphenicol), Treatment 1 (S. typhimurium infection + dose 100 mg/KgBB), Treatment 2 (*S. typhimurium* infection + dose 200 mg/KgBB), and treatment 3 (S. typhimurium infection + dose 400 mg/KgBB), and ethically feasible with a number 291/KEPK/UDS/VI/2022.

2.3 *Salmonella typhimurium* infection

Mice infection using *Salmonella typhimurium* bacteria with a concentration of $1x10^8$ cfu orally, after 3 days, mice are evaluated based on the texture of feces, and blood smear examination with giemsa staining to determine the presence of bacteria, blood is obtained through the tail.

2.4 Stool Examination

Stool examination is carried out 3 days after infection with *Salmonella typhimurium* bacteria. The examination is seen from the color of the stool, the consistency of the stool, and the mucus contained in the stool.

2.5 Giemsa Staining

Giemsa's staining is done by taking blood from the tail, then a thin blood smear is performed, the smear is waited for dry, after drying, then fixed using ethanol and wait for it to dry. Then drip the dye giemsa until it covers all the pusants and wait for 20 minutes. After 20 minutes, then rinsed using aquades and wait for them to dry. After drying, then observe whether there are bacteria in the blood or not.

2.6 Administration of Black Grape Extract Therapy

The administration of CMC Na, chloramphenicol, and extract (dose can be seen at point 3.2) is done orally or by mouth using sonde for 7 days.

2.7 Mencit necropsy

All mice from the treatment were then necropsied and further analyzed further. The mice are put into a container that has been dripped with chlorophyll until it does not move and the heart stops, then, surgery is performed and the spleen organs are taken for flowcytometric analysis.

2.8 Flowcytometric Analysis

The spleen that has been obtained, then washed and soaked in PBS volume 5 ml, the spleen that has been soaked is then grinding using the tip of the base of the syringe volume 3 ml, the scouring results are then filtered using wire and put into a

propylene tube volume of 15 ml to a certain volume (1: 3). Then centrifuged at a speed of 2500 rpm for 5 minutes with a temperature of 10°C, the centrifuged supernatant was discharged. Resuspended with PBS 1 ml then pipeting.

The result of the resuspension was introduced into a microtube volume of 1.5 ml by 50 µl. The centrifugation was carried out again at a speed of 2500 rpm, for 5 minutes with a temperature of 10°C, then discarded the centrifuged supernatant. The addition of 50 µl of sepsific antibody solution to the cells, namely $CD4^+$ and $CD8^+$ antibodies, was incubated for 20 minutes at a temperature of 4°C in a dark room. Finally, 400 µl of PBS was added and transferred it to the FCM cuvette for flowcytometric analysis.

2.9 Analysis of Results

The results of the $CD4^+$ and CD8+ expression analysis that have been obtained are then analyzed using the *SPSS for Windows* application. The test used is the anova test. In the anova test, if the significance value is below 0.05 ($p<0.05$) then continue to use the *post hoc* test (Tukey) to find out which concentration groups are different or find out the differences between the treatment groups.

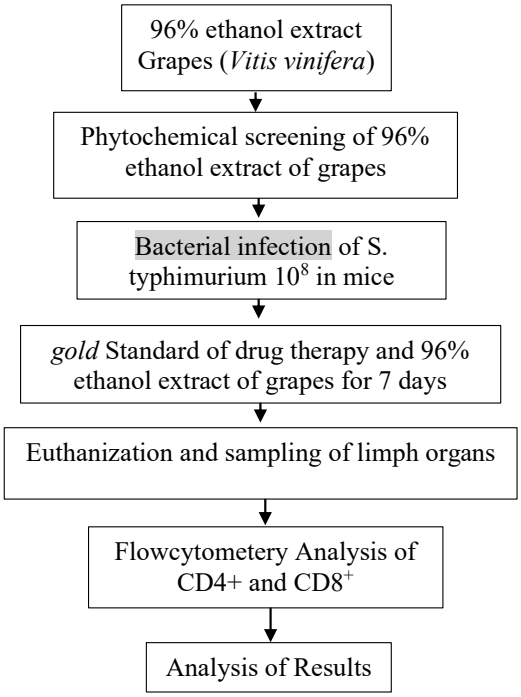


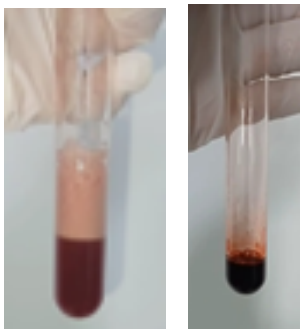
Figure 1. Research Chart

3. RESULTS AND DISCUSSION

Based on the research that has been carried out, several results were obtained, including:

3.1 Phytochemical Test of Black Grape Extract

Based on qualitative phytochemical analysis, secondary compounds in the form of saponins, tannins, and flavonoids were obtained (figure 2).



a b

c

Figure 2. The results of the phytochemical test of black grape extract (*Vitis Vinifera*). (a) Saponins, (b) Flavonoids, (c) Tannins.

Based on figure 2. Showed that the secondary metabolite compound saponin (a) formed foam or foam after the addition of aquades and shaking for 10 minutes. The flavonoid secondary metabolite compound (b) is red, and finally the tannin secondary metabolite compound (c) is blue, dark purple-black. This indicates that black grape extract contains secondary metabolite compounds in the form of saponins, flavonoids, and tannins, which can later act as immunomodulators of immune cells.

3.2 Stool Examination

After 3 days the mice were infected, then observations of the morphology of feces between positive mice compared to negative mice (figure 3) were carried out.

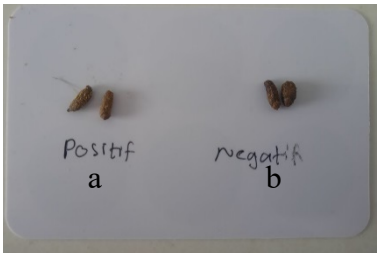
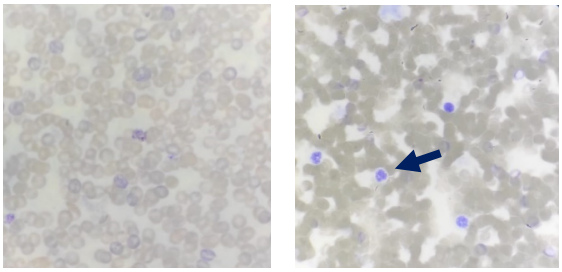


Figure 3. Differences in positive feces *Salmonella typhimurium* and negative. (a) Positive, (b) negative.

In **figure 3**, it is known that there are differences in color and consistency of the feces. Judging from the color, there is a change in color between positive and negative stools, in positive mices the color of feces is more fading or brown than warha feces of negative mice which are black. Then judging by the consistency, positive mice feces are more flabby and slimy compared to dense and hard negative mice feces.

3.3 Examination of Bacterimia with Giemsa Staining

In addition to conducting macroscopic examination, namely distinguishing the morphology of positive and negative mice feces, to ensure that the infection has spread systemically, giemsa's staining is carried out (figure 4).



a b

Figure 4. Giemsa coloring. (a) Negative *Salmonella typhimurium*, (b) Positive *Salmonella typhimurium*

In Figure 4, it can be seen that there is a difference in blood smears in negative mice and positive mice. After staining the blood smear and looking at it microscopically, in the negative mice (a) there is an erythrocyte formation but there is no *Salmonella typhimurium* bacteria.

However, in positive mice (b) there is a morphological formation of red blood cells (ertirocytes) around which there is *Salmonella typhimurium* bacteria (blue arrows), this indicates that there has been an

infection in mice and the bacteria has spread to the body through blood vessels.

3.4 CD4+ and CD8+ Examination Using Flowcytometry

After examination CD4+ and CD8+ using flowcytometry, the following results were obtained (figure 5).

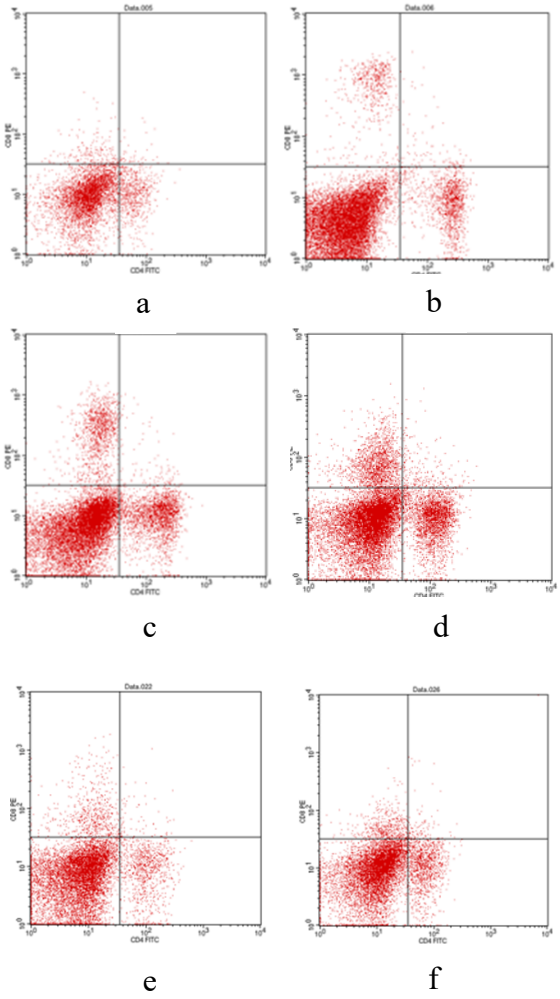


Figure 5. Flowcytometric examination results of CD4+ T cells and CD8+ T Cells. (a) Negative control, no treatment, (b) positive control (bacterial infection *salmonella typhimurium* + placebo), (c) standard control (bacterial infection *Salmonella typhimurium* + chloramphenocol therapy), (d) KP1 (bacterial infection *Salmonella typhimurium* + therapeutic dose 100 mg/KgBB), (e) KP2 (bacterial infection *Salmonella typhimurium* + therapeutic dose 200 mg/KgBB), (f) KP3 (bacterial infection *Salmonella typhimurium* + therapeutic dose 400 mg/KgBB).

Based on the figure above, it can be seen that there are differences in the expressions CD4 + and CD8 + for each group, which will be described in table 1 and table 2.

Table 1. CD4+ expression using flowcytometry method

No	K-	K+	K. S.	K.1	K.2	K.3
1	14,1	8,6	10,9	18,7	5,9	12,4
2	19,4	6,0	15,7	18,2	8,2	13,7
3	5,8	12,6	28.6	7,6	7,6	18,3
4	11,7	6,2	16,8	16,4	17,0	12,8
5	11,8	3,3	15,3	34,3	19,0	5,5
Average	12,6	7,3	14,7	19,0	11,5	12,5

Information:
K- = Negative control
K+ = Positive Conrols
K.S. = Standard Control
K.1 = KP1
K.2 = KP2
K.3 = KP3

Tabel 2. CD8+ expression using flowcytometric method

No	K-	K+	K. S.	K.1	K.2	K.3
1	9,3	3,5	15,6	10,6	1,1	5,1
2	15,6	2,2	4,9	10,3	5,2	5,2
3	2,6	5,4	8,9	5,8	3,7	8,4
4	3,3	5,4	7,2	4,2	9,4	6,9
5	3,3	0,6	7,6	20,3	8,9	1,2
Average	6,8	3,4	8,8	10,2	5,7	5,3

Keterangan:
K- = Negative control
K+ = Positive Conrols
K.S. = Standard Control
K.1 = KP1
K.2 = KP2

K.3 = KP3

The data is then carried out statistical analysis to find out whether there are noticeable differences between groups or not. Based on the analysis of statistical data using One Way Anova 95%, it shows that the expression of CD4 + and CD8 $^{+}$ does not differ markedly ($p>0.05$), so based on these statistical data there is no difference between each group from the negative, positive, standard, KP1, KP2, and KP3 groups.

However, biologically there are differences between each treatment, where in infected mice the expression of CD4 + and CD8 + has the lowest number of expressions compared to other groups, while for extract therapy shows extracts at doses of 100 mg / KgBB showing the highest expression of CD4 + and CD8 $^{+}$, however, in contrast to doses of 100 mb/KgBB, at doses of 200 mg/KgBB and 400 mg/KgBB showed a decrease in CD4+ and CD8^{+} expression (figure 6).

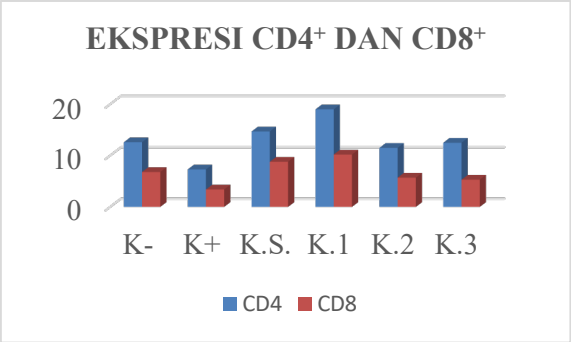


Figure 6. Differences in CD4+ and CD8^{+} expressions for each group

Salmonelosis is a disease caused by salmonella bacteria and is one of the world's problems. Salmonella bacteria also have the ability to resist antibiotics given or referred to as antibiotic resistance (Huang, 2021). one species of salmonella bacteria is Salmonella *typhimurium*.

Salmonella typhimurium is a rod-shaped bacterium and is gram-negative, in general this bacterium attacks the gastrointestinal part (Kaur and Gandhi, 2017). In addition, *Salmonella typhimurium* bacteria are bacteria used to model salmonella typhi infection in mice and rats, *Salmonella typhimurium* bacteria reach the highest number in the colon interacting with microbiota in the place (L., M. and J., 2020).

Infection with the bacterium *Salmonella typhimurium* in the colon can express *Type III Secretion System* 1 (T3SS1) which is encoded in *Salmonella Pathogenicity Island* 1 (SPI-1) and is an effector used to infect non-phagoitosis epithelial cells and phagocyte cells, for example, macrophages, as well as other cells, namely fibroblasts. When in the lumen, this bacterium will penetrate the intestinal wall to go to the lamina propia and tissues that have immunity cells, so that this bacterium will be carried to the nearest lymph nodes, namely the mesenteric lymph nodes and will later continue to the liver and spleen (Luk *et al.* , 2021) through blood vessels (Figure 4).

In the enterocyte area, *Salmonella typhimurium* bacteria are encapsulated and form an endocytic called *Salmonella-containing vacuole* (SCV), and affect the pH in the digestive tract, namely a decrease in pH. A decrease in pH leads to the cessation of T3SS1 and expresses T3SS2 from *Salmonella Pathogenicity Island* 2 (SPI-2), the effector T3SS2 plays a role in the replication of *S. tyhpimurium* bacteria (Luk *et al.* , 2021) . Replication that occurs in the intestinal part, especially the colon, causes damage to the liver and intestines (He *et al.* , 2021) .

The occurrence of intestinal damage and increased replication of *Salmonella typhimurium* bacteria in the intestinal part, induces a response from immune cells. In general, the mechanism of introduction of bacteria by immunity is played by *antigen precenting cells* (APC), one of which is dendritic cells. Dendritic cells will recognize bacteria through their receptors, namely *Toll-Like Receptors* (TLRs), one of which is TLR 4 which recognizes the structure of lipopolysaccharides (LPS) (Piccioli *et al.* ,

2022) and presenting it to $CD4^+$ T cells via MHC II thereby stimulating increased expression of $CD4^+$ T cells (Leone, Rees and Cain, 2018). However, in *Salmonella typhimurium* bacterial infections, these bacteria damage or inhibit the presentation of MHC II to $CD4^+$ T cells (Alix *et al.* , 2022) so that CD4+ T cells are not induced and decrease the expression of $CD4^+$ T cells when compared to normal (without infection) (figures 5a,5b), this event will lead to excessive systemic infection played by *Salmonella typhimurium* bacteria.

The confirmation of the presence of infection or a decrease in clinical symptoms of *Salmonella typhimurium* bacterial infection is to use antibiotics. However, *Salmonella typhimurium* or Salmonella sp. bacteria It is resistant to some antibiotics that are given or what is referred to as *multi-drug resistance* (MDR) (Debroy *et al.* , 2020) , one of which is chloramphenicol. However, based on the graph (figure 6) chloramphenicol administration can increase the expression of CD4+ T cells and CD8+ T cells in mice infected with *Salmonella typhimurium* bacteria.

The use of black aggur fruit extract aims to increase immunity and prevent an increase in resistance, through the antibacterial mechanism possessed by the extract, but in this discussion it will focus more on improving the immune system. Black grape extract is a plant that can grow in the tropics, one of which is in the Buleleng area of Bali. Black grape extract contains several phytochemicals including polyphenols, anthocyanins, flavonols, stilbenes, phenolic acids, proteins, fats, and vitamin C. Phytochemical content of black grape seeds includes prosianidin, gallic acid, epicatechin, catechin, quercetin, flavonol glycoside, resveratrol, anthocyanidin, caffeine acid, coumaric acid, koutaric acid, ferulic acid, ferturic acid, routine, Quercetin-3-β-D-glucoside, quercitrin, mirisetin, catechins, and epicatesin (Insanu *et al.* , 2021) . Black grape extract (*Vitis vinifera*) has flavonoid compounds that are the largest component in the skin and fruit, as well as protoantosianidin found in the seeds (Siswanto *et al.* , 2020) . The seeds of black grapes contain resveratrol (Soleymani *et al.* , 2019).

Protoantosianidin is a bioactive component found in fruits and vegetables. Protoantosianidin is derived from tannin compounds found in fruits and vegetables and has many benefits including metabolic syndrome regulation, immune cell modulation, cancer prevention, and nerve protection (Zhang *et al.* , 2023) (Bridson *et al.* , 2019) . Based on the function of immunity, that proantosianidin plays a role in increasing lymphocyte proliferation, NK cell cytotoxicity, $CD4^+/CD8^+$ ratio, production of interleukin 12 (IL-12) and interferon gamma (IFN-gamma) (Rauf *et al.* , 2019) .

Resveratrol is a component of polyphenols (Shaito *et al.* , 2020) which acts as an anti-tumor, and affects the activity of immune cells, especially anti-tumor immune cells (Mu and Najafi, 2021). Based on its immunological function resveratrol serves to induce the cytokine Interferon gamma (IFN-gamma), TNF-alpha and inhibits TGF-beta, and can stimulate the polarization of $CD4^+$ T cells (Chen and Musa, 2021). In addition, resveratrol also plays a role in increasing the ratio of $CD4^+$ T cells/$CD8^+$ T cells (Chen *et al.* , 2020) .

Based on these compounds, and based on the results obtained that proantosianidin and resveratrol or compounds from black grape extract (*Vitis vinifera*), play a role in the increase of $CD4^+$ T cells and $CD8^+$ T cells (figure 5d) when compared to other groups (Table 1 and Table 2). However, at medium to high doses, there is a decrease in the expression of $CD4^+$ T cells and $CD8^+$ T cells (figures 5e, 5f) when compared to low doses (figure 5d), this is because black grape extract (*Vitis vinifera*) has other anti-inflammatory compounds, one of which is quercetin.

Quercetin is a component of flavnoids that play a role in regulating immune cells,

anti-cancer, and anti-viral (Khazdair, Anaeigoudari and Agbor, 2021). In IBD disease research, the administration of quercetin can inhibit or decrease the activation of $CD4^+$ (Ju *et al.* , 2018) . However, it is necessary to conduct further research related to the decrease in the expression of $CD4^+$ T cells and $CD8^+$ T cells against *Salmonella typhimurium* bacterial infections treated using black grapes (*Vitis vinifera*).

4. CONCLUSION

Based on the data above, it can be concluded that giving black grape extract at a dose of 100 mg/KgBB can increase the expression of $CD4^+$ T cells and $CD8^+$ T cells, while black grape extract doses of 200 mg/KgBB and 400 mg/KgBB can reduce the expression of $CD4^+$ T cells and $CD8^+$ T cells.

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BIBLIOGRAPHY

Alix, E., Romina, T., Lisa, B., Sophie, M., Camilla, G., Ondrej, C., and David, H 2022. A genome-wide CRISPR screen identifies host proteins required for depletion of surface MHCII by Salmonella, *Molecular Immunology*, 150, p. 6. doi: https://doi.org/10.1016/j.molimm.2022.05.031.

B Bridson, J. H., Marion, S., Ibrar H., Vincent, B., Meeta, P., and Kate, P. 2019. Hydroxyalkylation of condensed tannins: Comparison of proanthocyanidin extraction process and epoxide chain length on physicochemical properties, *Industrial Crops and Products*, 140, p. 111618. doi: https://doi.org/10.1016/j.indcrop.2019.111618.

Chen, L. and Musa, A. E. 2021. Boosting immune system against cancer by resveratrol, *Phytotherapy Research*, 35(10), pp. 5514–5526. doi: https://doi.org/10.1002/ptr.7189.

Chen, M., Qiuting, F., Xu, S., Abaidullah, M., Renyong, J., Yuanfeng, Z., Lizi, Y., Lixia, L., Changliang, H., Gang, Y., Cheng, L., Xiaoxia, L., Juang, H., Min, C., and Zhongqiong, Y. 2020. Preparation of resveratrol dry suspension and its immunomodulatory and anti-inflammatory activity in mice, *Pharmaceutical Biology*, 58(1), pp. 8–15. doi: 10.1080/13880209.2019.1699123.

Debroy, R., Sravan, K. M., Aniket, N., Anand, A., and Sudha, R. 2020. Gene interaction network studies to decipher the multi-drug resistance mechanism in Salmonella enterica serovar Typhi CT18 reveal potential drug targets, *Microbial Pathogenesis*, 142, p. 104096. doi: https://doi.org/10.1016/j.micpath.2020.104096.

Ehuwa, O., Jaiswal, A. K. and Jaiswal, S. 2021. Salmonella, Food Safety and Food Handling Practices, *Foods*. doi: 10.3390/foods10050907.

El-Desouky, W., Hanafi, A. and Abbas, M. M. 2017. Radioprotective effect of green tea and grape seed extracts mixture on gamma irradiation induced immune suppression in male albino rats, *International Journal of Radiation Biology*, 93(4), pp. 433–439. doi: 10.1080/09553002.2016.1254834.

Elbediwi, M., Yangting, T., Dawei, S., Hazem, R., Yaohui, X., Sihong, X., Yan, L., and Min, Y. 2021. Genomic Investigation of Antimicrobial-Resistant Salmonella enterica Isolates From Dead Chick Embryos in China, 12(August). doi: 10.3389/fmicb.2021.684400.

He, Y., Hammed, A., Qihang, H., Chaoyong, L., Jiaqi, L., Yujiao, L., Qiuyu J., Hori, M., and Bingkun, Z. 2021. Genistein Inhibits Colonic Goblet Cell Loss and Colorectal Inflammation Induced by Salmonella Typhimurium Infection, *Molecular Nutrition & Food Research*, 65(16), p. 2100209. doi: https://doi.org/10.1002/mnfr.202100209.

Huang, F. 2021. The Interleukins Orchestrate Mucosal Immune Responses to Salmonella Infection in The Intestine, *Cells,* 10(12): 3492. https://doi.org/10.3390/cells10123492.

Insanu, M., Hana, K., Hegar, P., and Irda, F. 2021. Phytochemical compounds and pharmacological activities of vitis vinifera L.: An updated review, *Biointerface Research in Applied Chemistry*, 11(6), pp. 13829–13849. doi: 10.33263/BRIAC115.1382913849.

Jahan, F., Suresh. V.C., Sumitha, S., Lebaka, V.R., Maheswaran, S., and Lee, S.Y. 2022. The Complex Mechanism of the Salmonella typhi Biofilm Formation That Facilitates Pathogenicity : A Review'. *Int. J. Mol. Sci*, 23(12), 6462; https://doi.org/10.3390/ijms23126462.

Jassim, M. and Obead, M. 2022. Integron-Associated Antibiotic Resistance in Salmonella typhi, 77(2), pp. 771–777. doi: 10.22092/ARI.2021.356953.1944.

Ju, S., Yan, G., Ping, L., Xinxin, T., haiyan, W., Xiaocui, Z., and Songguang, J. 2018. Dietary quercetin ameliorates experimental colitis in mouse by remodeling the function of colonic macrophages via a heme oxygenase-1-dependent pathway, *Cell Cycle*, 17(1), pp. 53–63. doi: 10.1080/15384101.2017.1387701.

Kaur, D. and Gandhi, S. 2017. Salmonella Typhimurium Adhesin OmpV Activates Host Immunity To Confer Protection against Systemic and Gastrointestinal Infection in Mice, *Microbial Immunity and Vaccines*, 89(8). https://doi.org/10.1128/IAI.00121-21

Khazdair, M. R., Anaeigoudari, A., and Agbor, G. A. 2021. Anti-viral and anti-inflammatory effects of kaempferol and quercetin and COVID- 2019 : A scoping review, 11(March), pp. 327–334. doi: 10.4103/2221-1691.319567..

L., R. A. W., M., T. R. and J., B. A. 2020. Salmonella versus the Microbiome, *Microbiology and Molecular Biology Reviews*, 85(1). doi: 10.1128/MMBR.00027-19.

Leone, D. A., Rees, A. J. and Kain, R. 2018. Dendritic cells and routing cargo into exosomes, *Immunology & Cell Biology*, 96(7), pp. 683–693. doi: https://doi.org/10.1111/imcb.12170.

Luk, C. H., Camila, V., Magdalena, G., Lea, S., Parrine, B., Yuen-Yang, C., and Ade, J.E. 2021. Salmonella enters a dormant state within human epithelial cells for persistent infection, *PLOS Pathogens*, 17(4). https://doi.org/10.1371/journal.ppat.1009550.

Mahari, S. and Gandhi, S. 2022. Recent Advances in Electrochemical Biosensors for the Detection of Salmonellosis : Current Prospective and Challenges, *Biosensors*. 12(6). https://doi.org/10.3390/bios12060365

Malaguarnera, L. 2019. Influence of resveratrol on the immune response, *Nutrients*, 11(5), pp. 1–24. doi: 10.3390/nu11050946.

Mu, Q. and Najafi, M. 2021. Resveratrol for targeting the tumor microenvironment and its interactions with cancer cells, *International Immunopharmacology*, 98, p. 107895. doi: https://doi.org/10.1016/j.intimp.2021.107895.

Piccioli, D., Renzo, A., Valentina, M., Vanessa, A., Martina, C., Maria, G.A., Omar, R., Francesca, N., Alessandra, A., Erika, B., and Francesca, M. 2022. Antigen presentation by Follicular Dendritic cells to cognate B cells is pivotal for Generalised Modules for Membrane Antigens (GMMA) immunogenicity', *Vaccine*, 40(44), pp. 6305–6314. doi: https://doi.org/10.1016/j.vaccine.2022.09.034.

Rauf, A., Muhammad, I., Tareq, A., Iahtisham, U., Seema, P., Xiandao, P., Saima, N., Ana, S.S., Farhan, S., Hafiz, A., and Rasul, S. 2019. Biomedicine & Pharmacotherapy Proanthocyanidins : A comprehensive review, 116. doi: 10.1016/j.biopha.2019.108999.

Shaito, A., Anna, M.P., Nadin, Y., Hiba, H., Sarah, H., Dalal, A., Anjud, A., Wael, M.A., Ali, H.D., Gheyath, K.N., and Gianfranco, P. 2020. Potential Adverse E ff ects of Resveratrol : A Literature Review'. *Int. J. Mol. Sci*., 21(6); https://doi.org/10.3390/ijms21062084

Siswanto, F. M., Risha, C.P., Boedi, P.Y., and

Alex, P., 2020. Ethanolic extract of black grapes (Vitis Vinifera) ameliorates overtraining-induced pancreatic β-cells and muscle damage, *Jurnal Riset Veteriner Indonesia (Journal of The Indonesian Veterinary Research)*, 4(2). doi: 10.20956/jrvi.v4i2.9688.

Soleymani, S., Amin, I., Fariba, N., tarun, B., Sudipta, R., Zahra, A., Saeideh, M., and Mohammad, H.F., 2019. Implications of grape extract and its nanoformulated bioactive agent resveratrol against skin disorders, *Archives of Dermatological Research*, 311(8), pp. 577–588. doi: 10.1007/s00403-019-01930-z.

Talukder, M., Md, S.I., Samina, I., Md. A.S., Fatimah, M.B., Md, N., Md, B.R., Md, T.R., and Mohammad F.R.K. 2021. Detection of multidrug resistant Salmonella spp . from healthy and diseased broilers having potential public health significance, Journal of Advanced Biotechnology and Experimental Therapeutics, 4(2). doi: 10.5455/jabet.2021.d125.

Wójcicki, M., Olga, S., Jamila, J.D., Paulina, S., Monika, A., Marek, L.R., Barbara, S., and Edyta, J. 2021). Transcriptional Regulation of the Multiple Resistance Mechanisms in Salmonella—A Review', *Pathogens*, 10(7). doi: 10.3390/pathogens10070801.

Yadav, J. 2020. Innate Activation of IFN-γ—iNOS Axis During Infection With Salmonella Represses the Ability of T Cells to Produce IL-2, *Frontiers in Immunology*, 11. doi: 10.3389/fimmu.2020.00514.

Zhang, T. 2018. polyphenols extracted from Ilex latifolia Thunb', *Royal Society of Chemistry*, 8. doi: 10.1039/c7ra13569f.

Zhang, X., Xunyu, S., Xiaosong, H., Fang, C., and Chen, M. 2023. Health benefits of proanthocyanidins linking with gastrointestinal modulation: An updated review', *Food Chemistry*, 404. doi: https://doi.org/10.1016/j.foodchem.2022.134596.