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Effectiveness of Herbal Medicine Mangosteen Peel Extract (*Garcinia mangostana*) to Prevent Free Radicals Occurrence and Decrease in Hemoglobin Levels in the Blood Caused by Diabetes Mellitus

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Abstract

Diabetes mellitus is associated with a decrease in hemoglobin levels. The relationship between the 2 is inversely proportional. When blood sugar levels increase, it triggers glycation, enhancing xanthine oxidase activity and free radical formation. This phenomenon causes reactive oxygen species (ROS) to emerge and reduces oxygen in the blood, decreasing hemoglobin levels. This study aims to analyze the effectiveness of mangosteen peel extract (*Garcinia mangostana*) in preventing the formation of free radicals and reducing hemoglobin levels in the blood due to diabetes mellitus. This study was conducted using 195 experimental mice (*Mus musculus*). Diabetes mellitus was induced in the mice by injecting streptozotocin (dissolved in 0.1 M citrate buffer, pH 4.5) for 7 days. The dose of streptozotocin administered to create a pre-diabetes group was 10 mg/kg BW (PN and PO) and 30 mg/kg BW to create a diabetes group (DN and DO). The injection route used for streptozotocin was the intraperitoneal (IP) method. Mangosteen peel extract (*Garcinia mangostana*) was administered orally at doses of 0.86, 1.86, 2.86, 3.86 and 4.86 mg/mL. The mangosteen peel extract treatment was carried out for 14 days. Measurements in the mice included tests for hemoglobin levels, types of free radicals, relative levels of free radicals, and histopathological observations using Scanning Electron Microscopy (SEM). The free radicals were superoxide anion ($*O_2^-$) and hydroxyl radical ($OH*$). The results showed that mangosteen peel extract (*Garcinia mangostana*) effectively prevented the decrease in hemoglobin levels and broke the chain of free radical formation. Hemoglobin levels in the diabetes group increased by 61.5 %, while the pre-diabetes group increased by 24 %, approaching the hemoglobin level of the control group at 19 ± 1.2 g/dL. The most effective dose of mangosteen peel extract (*Garcinia mangostana*) was 2.86 mg/mL. Mangosteen peel contains xanthone, which can counteract free radicals through single electron transfer (SET) and hydrogen atom transfer (HAT).

Keywords: Diabetes mellitus, Free radicals, *Garcinia mangostana*, Hemoglobin, Streptozotocin

Introduction

Diabetes mellitus can increase the formation of free radicals through many different pathways. Based on several previous *in vivo* and *in vitro* studies, there are at least 10 types of mechanisms for free radical formation during chronic hyperglycemia [1]. These mechanisms include mitochondrial mechanisms, disabling of the cellular antioxidant defense system (ADS), glucose autoxidation, glycation and associated pathways, lipid peroxidation, activation of free radical-generating enzymes, the polyol (sorbitol) pathway, protein kinase-C (PKC) isoforms, the hexosamine pathway, and redox state changes [2,3]. In addition to these 10 mechanisms, the Fenton reaction, metabolic changes, and the accumulation of ketone bodies can also contribute as alternative routes, interacting through more complex molecular mechanisms, potentially causing induction or inhibition of one another, either directly or indirectly. Excessive free radical formation is followed by

oxidative stress, which can lead to various other diabetes complications [4,5].

Hemoglobin is a tetrameric protein in red blood cells useful for binding oxygen (O_2) and is distributed throughout the body [6]. The hemoglobin molecule consists of heme (iron) and globin polypeptide chains. The normal human metabolism utilizes most of the consumed glucose to be converted into energy in the form of ATP through aerobic processes. This mechanism requires an oxygen supply from the mitochondria to support the activity of pro-oxidant enzymes. These pro-oxidant enzymes can produce reactive oxygen species (ROS) but only for intracellular signaling and several physiological processes essential to the body [7]. However, if the ROS levels are excessive, the resulting impact can harm health, causing oxidative stress and decreasing hemoglobin levels [8,9].

Hyperglycemia can trigger additional metabolic pathways, such as the protein kinase C, polyol, and hexosamine pathways, as well as the formation of advanced glycation end products (AGEs), which are proven to damage the cellular structure of mitochondria [10]. Damage to the mitochondria can disrupt the electron transport chain (ETC) and reduce ATP production through cellular respiration. Low ATP levels will increase xanthine oxidase activity, making xanthine oxidase a catalyst for ROS formation by reducing oxygen content in the blood. The more oxygen that is reduced to ROS, the lower the hemoglobin levels in the blood [8].

The common treatments for diabetes mellitus include insulin injections or chemical medications such as metformin. However, the use of chemical medications and insulin injections can lead to dangerous side effects [11-13]. Anti-hyperglycemic drugs may cause side effects such as digestive issues, hypoglycemia, and weight gain. Previous research has shown the potential prevalence of anemia due to diabetes mellitus therapy, leading to a deficiency of red blood cells or healthy hemoglobin needed to transport oxygen throughout the body. The side effects of anti-hyperglycemic drugs are highly complex. They can be classified based on the type of treatment received (e.g., metformin or insulin injections), the duration of diabetes (over 5 years), nutritional status, stress level, age, and the possibility of taking additional medications. Given these issues, it is necessary to develop a type of natural treatment that is safer, simpler, side effect-free, affordable, and accessible to all layers of society [11].

Mangosteen (*Garcinia mangostana*) is a tropical plant widely found and cultivated across Southeast Asia, including Indonesia, Malaysia, Thailand, and Myanmar [14]. The mangosteen peel, still considered waste, has very limited use. This study utilizes mangosteen peel extract as an antioxidant or herbal remedy to prevent the decrease of hemoglobin levels in the blood caused by diabetes mellitus. Mangosteen peel was chosen over other plants because it is readily available and rich in antioxidants such as vitamin C, phenols, flavonoids, tannins, mangostin, garsin, tannins, and xanthones [15-17]. The xanthone content in mangosteen is predominantly found in the peel, with each gram containing approximately 107 ± 0.76 mg of xanthones [14]. The types of xanthones in mangosteen peel, including *α-mangostin*, *β-mangostin*, *γ-mangostin*, *gartanin*, *and 8-deoxygartanin*, have properties that are antidiabetic, antioxidant, antibacterial, anticancer, antifungal, antihistamine, anti-inflammatory, and can even be used in HIV therapy [18-20]. No prior research has examined the correlation between diabetes mellitus and reduced hemoglobin levels alongside the use of herbal remedies to address this issue. This study aims to analyze the effectiveness of mangosteen peel extract (*Garcinia mangostana*) in preventing the formation of free radicals and the decrease in hemoglobin levels in the blood due to diabetes mellitus.

Materials and methods

Experimental animal treatment

This study used 195 male mice (*Mus musculus*) of the BALB/c strain, with a body weight of 28 ± 2 g and an age of 13 weeks. All mice used were standard laboratory mice obtained from Brawijaya University (approval number: 090-KEP-UB 2023, dated 07.07.2023). Before receiving streptozotocin injections, the mice were acclimatized for 7 days in a controlled cage environment maintained at a stable temperature of 25 ± 1 °C, humidity of 48 ± 8 %, with a 12-hour dark/light cycle in the Biophysics Laboratory. After the acclimatization phase, the mice were randomly divided into 13 groups, as shown in **Table 1**.

Table 1 Scheme for dividing groups of mice.

Treatment group	Code	Number of mice	Streptozotocin dosage	Mangosteen peel dosage
Control	K	15 mice	-	-
Pre-diabetes without medication mangosteen peel extract	PN	15 mice	10 mg/kgBW	-
Pre-diabetes with mangosteen peel extract medication	PO 1	15 mice	10 mg/kgBW	0.86 mg/mL
	PO 2	15 mice	10 mg/kgBW	1.86 mg/mL
	PO 3	15 mice	10 mg/kgBW	2.86 mg/mL
	PO 4	15 mice	10 mg/kgBW	3.86 mg/mL
	PO 5	15 mice	10 mg/kgBW	4.86 mg/mL
Diabetes mellitus without medication mangosteen peel extract	DN	15 mice	30 mg/kgBW	-
Diabetes mellitus with mangosteen peel extract medication	DO 1	15 mice	30 mg/kgBW	0.86 mg/mL
	DO 2	15 mice	30 mg/kgBW	1.86 mg/mL
	DO 3	15 mice	30 mg/kgBW	2.86 mg/mL
	DO 4	15 mice	30 mg/kgBW	3.86 mg/mL
	DO 5	15 mice	30 mg/kgBW	4.86 mg/mL

Diabetes mellitus was induced in the mice by injecting streptozotocin (dissolved in 0.1 M citrate buffer, pH 4.5) for 7 days. The streptozotocin dose administered to create a pre-diabetes group was 10 mg/KgBW (PN and PO) and 30 mg/kg BW for the diabetes group (DN and DO). Streptozotocin was administered using the intraperitoneal (IP) injection method once daily. The mice were given a 10 % dextrose solution after the streptozotocin injection to prevent sudden hypoglycemia. Control group mice did not receive streptozotocin injections or mangosteen peel extract and were only given the same diet as all other groups, consisting of starch and cellulose-based food. The mangosteen peel extract (*Garcinia mangostana*) was obtained in pre-extracted form from a pharmaceutical store and administered to the mice orally once daily for 14 days [21].

Measurement of blood sugar level and hemoglobin

Blood glucose levels were measured daily after all mice were fasted for 12 h. Blood glucose measurement was done using a rapid-check glucometer with an accuracy of ≥ 90 %, as specified in the device's specifications. Hemoglobin levels were also measured using a rapid-check hemoglobin device with an accuracy of ≥ 90 %, as stated in the device's specifications. Both measurements were conducted continuously until the end of the experiment.

Measurement of free radical

Free radical testing uses Electron Spin Resonance (ESR) at 13 - 130 MHz with a current of 0.2 A. Before the tool is used, ESR is calibrated first using a DPPH calibrator. The type of free radical will be known based on the g-factor value through the equation;

$$g = \frac{hf}{\mu_B B} \quad (1)$$

μ_B = Bohr magneton ($\mu_B = 9.274078\times10^{-24}$ Am^2)
h = Plank's constant ($h = 6.625\times10^{-34}$ Ws^2)
f = Resonant frequency (Hz)
B = External magnetic field (T)

$$B = \mu_0\left(\frac{4}{5}\right)^{\frac{3}{2}}\frac{n}{r}\,I \quad (2)$$

$\mu_0 = 1.2566\times10^{-6}$ Vs/Am
n = Number of Helmholtz (n = 320)
r = Helmholtz coil radius (r = 6.8 cm)
I = Current in Helmholtz coil (A)

Histopathological observation

To observe the effectiveness of herbal medicine mangosteen peel extract (*Garcinia mangostana*) in preventing a decrease in hemoglobin levels in the blood caused by diabetes mellitus, histopathological observations of blood cells and hemoglobin measurements were carried out for all treatments. Histopathological observations were carried out using a Scanning Electron Microscope (SEM). SEM preparations are made through cryofixation, fixation, dehydration, embedding, sectioning, staining, and finally, freeze-fracture. Next, preparations are placed on the object table to be observed. Histopathological observations of blood were carried out with a magnification of 10,000 times.

Statistical analysis

Analysis of variance (ANOVA) and Pearson correlation were used to assess significance and differences between groups. Calculations were performed using Statistical Software 13.0 (10). An ANOVA test with a p-value < 0.05 was assumed to indicate statistical significance. A correlation coefficient of $r = 1$ indicates a perfect positive relationship, $r = -1$ indicates a perfect negative relationship, while $r = 0$ indicates no linear relationship between the 2 variables.

Limitations

This study only conducted measurements of blood sugar levels and hemoglobin using a rapid check with an accuracy of ≥ 90 %, as stated in the device specifications. We did not compare the device's measurements with multiple methods. Histopathological observations were only made using a Scanning Electron Microscope (SEM), and we did not perform observations using a light microscope or Complete Blood Count (CBC) to examine blood size, color, and abnormalities. This study did not observe changes in body weight, collect additional samples, or measure other physiological parameters.

Results and discussion

Results

Based on the measurement results, it was found that the injection of streptozotocin at a dose of 30 mg/kgBW successfully induced diabetes mellitus with an average blood glucose level of 373 ± 6 mg/dL (DN), while the injection of streptozotocin at a dose of 10 mg/kgBW successfully induced pre-diabetes with an average blood glucose level of 178 ± 4 mg/dL (PN). The administration of mangosteen peel extract reduced blood glucose levels, with the most significant effect observed at the third dose (**Figure**

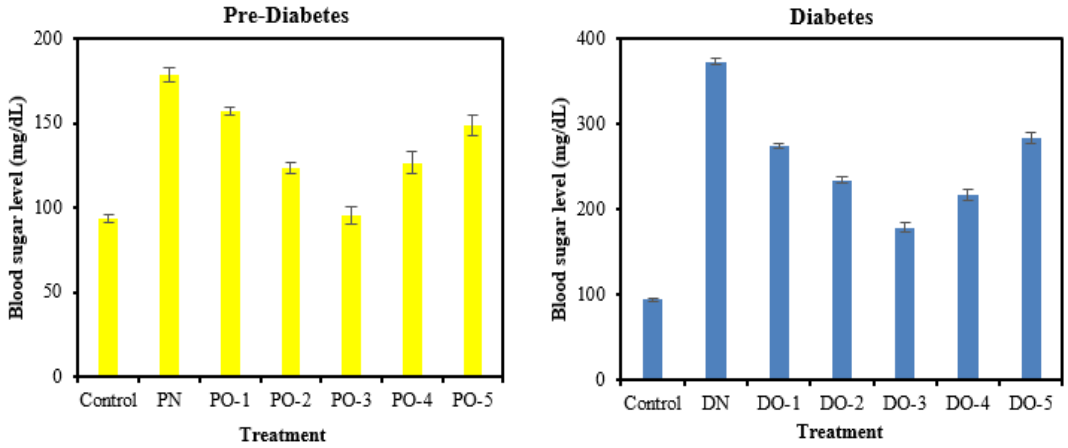

Figure 1 Graph of blood sugar levels in each treatment.

Our findings indicate that hemoglobin levels in diabetes mice (DN) are lower than in pre-diabetes mice (PN) and healthy mice (K), as shown in **Figure 2**. Low hemoglobin levels remain unchanged if the diabetes (DN) and pre-diabetes (PN) conditions persist. Based on statistical analysis, the blood glucose and hemoglobin levels in the pre-diabetes mellitus group showed a p-value of 5×10^{-7} with a correlation coefficient of $r = -0.92$. In the diabetes mellitus group, the p-value was 2×10^{-5} with a correlation coefficient of $r = -0.97$. These results indicate a statistically significant difference between blood glucose and hemoglobin levels.

These findings also clarify that the variation in blood glucose and hemoglobin levels across the different treatments is not due to chance but reflects a meaningful difference. The very small *p*-value (less than 0.05) demonstrates that these results are highly statistically significant, making it highly likely that this negative relationship is genuine and not merely coincidental. The correlation coefficients of r = −0.92 and −0.97 indicate a strong negative correlation between blood glucose and hemoglobin levels. This negative correlation means that hemoglobin levels tend to decrease almost perfectly as blood glucose levels increase. Hemoglobin levels could increase after mice were orally given mangosteen peel extract (*Garcinia mangostana*) for 14 days. The increase mice hemoglobin levels for pre-diabetes group was 9.4 % (PO-1), 13.7 % (PO-2), 24 % (PO-3), 1.4 % (PO-4) and 1.5 % (PO-5) while in diabetes mellitus group increase in hemoglobin was 14.3 % (DO-1), 36.5 % (DO-2), 61.5 % (DO-3), 43.7 % (DO-4) and 22.1 % (DO-5). A percentage increase in hemoglobin levels proves that mangosteen peel extract (*Garcinia mangostana*) can restore and increase mice hemoglobin levels when they experience pre-diabetes and diabetes mellitus.

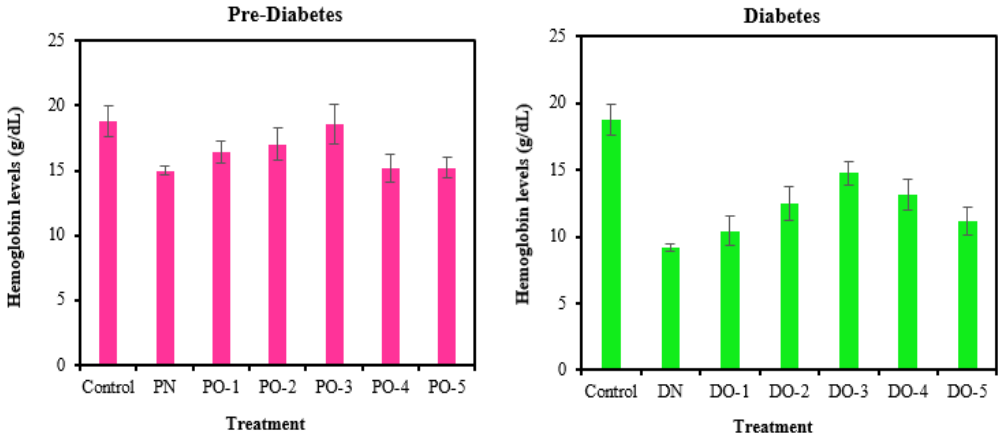

Figure 2 Graph of hemoglobin levels in mice for each treatment with mangosteen peel extract (*Garcinia mangostana*).

Based on variations in drug doses, it is known that the most significant and effective drug dose for increasing hemoglobin levels is a dose of 2.86 mg/mL. Hemoglobin measurement data also explains that overdose will reduce the effectiveness of the drug in treating diabetes mellitus, as shown by the PO-4, PO-5, DO-4, and DO-5 groups. Events in pre-diabetes mice PO-4 and PO-5 groups show that the drug's ability has reached a saturation point and can no longer increase blood hemoglobin levels. In the case of DO-4 and DO-5 groups, it also shows that when an optimal dose of the drug is exceeded, the effect of the drug is no longer a cure but instead worsens the impact of diabetes mellitus. Diabetes mellitus can trigger the formation of free radicals through various mechanisms. An excess of free radicals has the potential to cause oxidative stress, which can lead to a chain of events that reduces oxygen content and lowers hemoglobin levels in the blood [22-24].

Oxidative stress conditions can occur when the number of free radicals in the blood exceeds the limit the body can tolerate [25]. **Figure 3** shows the free radicals' relative levels in each group of mice. Free radicals' relative levels in the blood are higher when the concavity of the Lissajous curve displayed by the Electron Spin Resonance (ESR) unit is wider and steeper. A wide and steep Lissajous curve shows that the measured sample contains many unpaired electrons that experience magnetic resonance due to providing an external magnetic field from the Helmholtz coil [26].

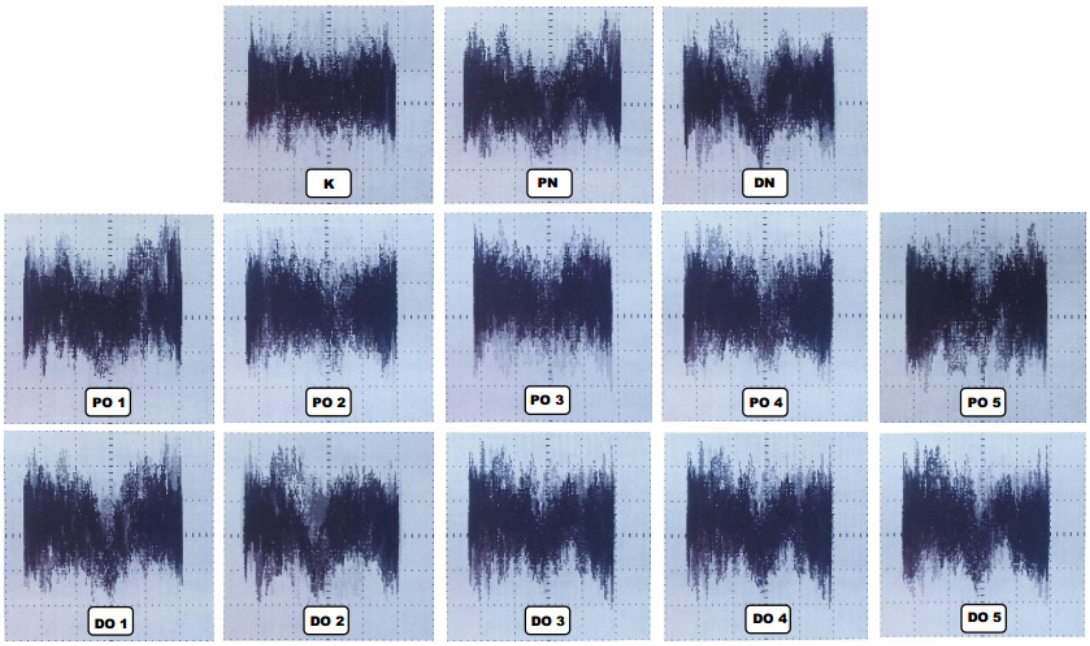

Figure 3 Measuring results of free radicals' relative levels using Lissajous curve (ESR).

The accumulation graph of free radicals' relative levels is shown in **Figure 4**, wherein control treatment, only superoxide anion free radicals ($*O_2^-$) were found at 0.18 ± 0.04 A.u. The discovery of the hydrogen peroxide compound (H_2O_2) of 0.22 ± 0.05 A.u is not a free radical but has properties similar to free radicals because it is very reactive to lipid molecules in the body.

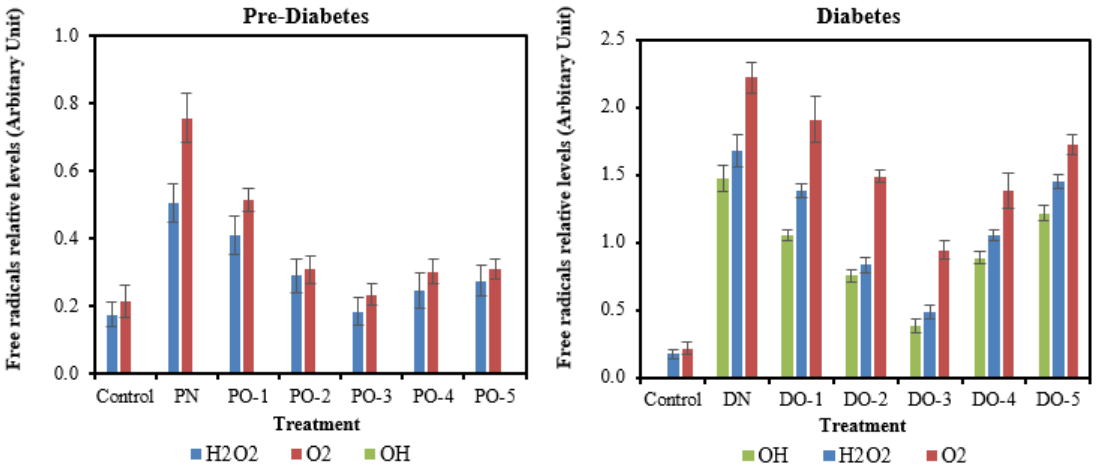

Figure 4 Accumulation graph of free radicals' relative levels in each treatment with mangosteen peel extract (*Garcinia mangostana*).

Free radical relative levels in the control group were very low and still tolerable by the body. Free radicals' relative levels increased during pre-diabetes and diabetes mellitus. These levels could be reduced after mice received treatment with mangosteen peel extract (*Garcinia mangostana*). ESR identification results revealed 1 type of free radical in the pre-diabetes group, namely superoxide anion ($*O_2^-$) with hydrogen peroxide molecules (H_2O_2), while in diabetes mellitus group, 2 types of free radicals were found, namely superoxide anion ($*O_2^-$) and hydroxyl radicals ($OH*$) with hydrogen peroxide molecules (H_2O_2). All free radicals identified are oxidative free radicals in the Reactive Oxygen Species (ROS) category [27].

Oxidative free radicals are highly reactive towards biological tissue components such as proteins, nucleic acids, lipids, and enzymes [28,29]. Free radical measurement results were highly correlated with hemoglobin level tests.

The statistical analysis results for the pre-diabetes group for the type of free radical ($*O_2^-$) showed a *p*-value of 1.3×10^{-7} with a correlation coefficient of r = 0.88. The molecule (H_2O_2) had a *p*-value of 1.2×10^{-7} with a correlation coefficient of r = 0.93. In the diabetes mellitus group, the free radical ($*O_2^-$) had a *p*-value of 1.3×10^{-5} with a correlation coefficient of r = 0.97, the free radical ($OH*$) had a *p*-value of 1.3×10^{-5} with a correlation coefficient of r = 0.97, and the molecule (H_2O_2) had a *p*-value of 1.3×10^{-5} with a correlation coefficient of r = 0.95. All statistical analysis results show significant differences, indicating a positive correlation. The results obtained are very likely genuine and not merely coincidental.

Impact observation of administering mangosteen peel extract (*Garcinia mangostana*) on blood cell morphology was carried out using a Scanning Electron Microscope (SEM) in each treatment group. The results of blood histopathological observations with a magnification of 10,000 times showed changes in morphology and cell membrane integrity in the groups that received and did not receive mangosteen peel extract (*Garcinia mangostana*) (**Figure 5**). SEM observation showed that the surface of the control group's red blood cells (RBC) and white blood cell (WBC) membranes were still clearly visible, and no degradation or structural changes were found in cell walls. In the control group, no significant damage was found. RBC and WBC in the control group were still neatly arranged with a clear shape. RBC has a slightly concave spherical shape with a diameter of ± 8 μm, while WBC has a spiny ball shape [30]. Pre-diabetes mellitus group showed changes in cell wall lines that were irregular and looked slightly faded. Changes in shape that occur are not uniform in all parts but can reflect the occurrence of cell wall degeneration when pre-diabetes mellitus occurs [31]. For the diabetes mellitus group, there were clear and even changes in cell wall lines almost throughout the visual field.

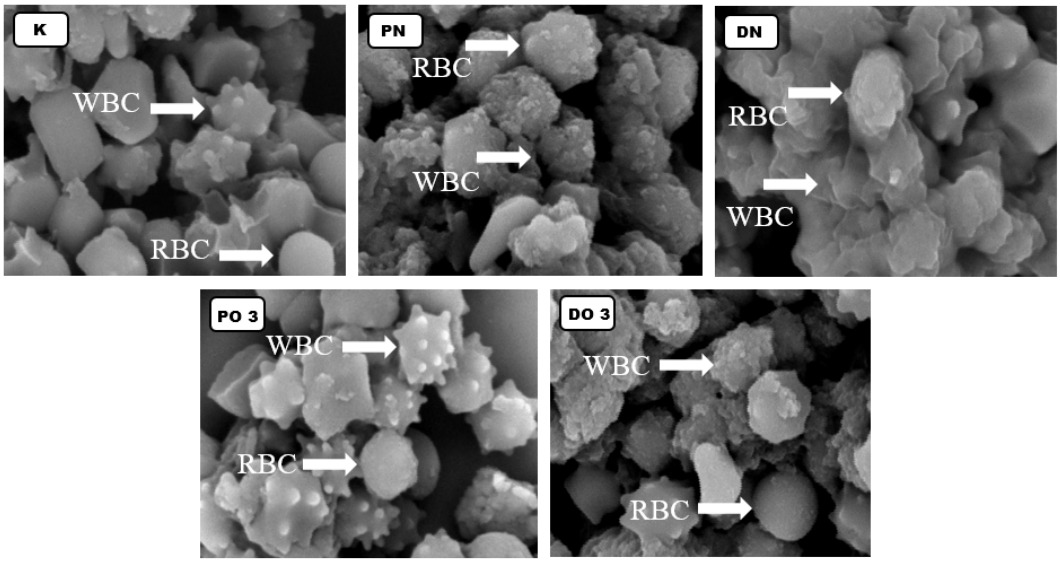

Figure 5 Normal blood cell morphology (K), pre-diabetes without medication (PN), diabetes mellitus without medication (DN), pre-diabetes with medication 2.86 mg/mL (PO 3), and diabetes mellitus with medication 2.86 mg/mL (DO 3).

Cell walls become irregular, making it difficult to distinguish between red blood cells and white blood cells. Cell walls that are difficult to differentiate indicate that blood cells have undergone fragmentation or lysis [32]. Morphological similarities that occur prove that the cell membrane has lost cytoskeleton stability and membrane integrity [33,34]. Drug administration to pre-diabetes and diabetes mellitus groups showed good changes in cell deformability. Giving the drug at a dose of 2.86 mg/mL was able to restore the shape and size of cells in the pre-diabetes group to that of the control group, while administering 2.86 mg/mL of the drug in

diabetes mellitus group was able to make shape and size of cells become like those pre-diabetes group. Red blood cell (RBC) structure changes show a strong negative correlation with hemoglobin levels in pre-diabetes and diabetes groups. The correlation coefficient in the pre-diabetes group shows an r-value of −0.93 with a *p*-value of 1.5×10^{-6}, while in the diabetes group, it shows an r-value of −0.97 with a *p*-value of 1.3×10^{-4}. These results indicate that the greater the changes in RBC structure, the more significant the decrease in hemoglobin levels. The relationship between RBC structural changes and hemoglobin levels is statistically significant. Furthermore, the relationship between RBC structural changes and hemoglobin levels strongly correlates with diabetes mellitus conditions. As blood glucose levels increase, RBC structural changes also intensify. The relationship between diabetes conditions and RBC structural changes is positive, with a correlation coefficient of r = 0.95 for both pre-diabetes and diabetes groups.

Discussion

Diabetes mellitus can occur due to uncontrolled increases in blood sugar levels. Blood sugar levels in the body are naturally controlled by insulin. Insulin is an agent that controls blood glucose levels by increasing glucose uptake into tissues [35]. Smooth glucose uptake will result in metabolism that can convert glucose into energy and be stored as glycogen or triglycerides. Inhibited glucose uptake causes blood glucose levels to rise and causes hyperglycemia [36,37]. Hyperglycemia conditions make sugar molecules more easily bound to proteins through a non-enzymatic reaction known as glycation. Glycation can cause damage to various cellular structures, including mitochondria [36,38,39]. Mitochondrial damage will disrupt the electron transport chain (ETC) and decrease ATP production cellular respiration process. Low ATP leads to increased xanthine oxidase activity [40,41]. Xanthine oxidase will then become a catalyst for the formation of ROS by accelerating oxygen reduction through the following stages;

a) Formation of superoxide anions ($*O_2^-$) or negatively charged single radicals result from oxygen reduction in the blood through oxidase reactions [42].

$$O_2 + e^- \longrightarrow *O_2^- \; Superoxide\ anion \quad (3)$$

b) Superoxide anion ($*O_2^-$) is then converted to hydrogen peroxide (H_2O_2). Hydrogen peroxide is formed from $*O_2^-$ Radicals with the help of enzyme superoxide dismutase (SOD) [27,43].

$$*O_2^- + e^- + 2H^+ \longrightarrow H_2O_2 \; Hydrogen\ peroxide \quad (4)$$

c) With the help of the aconitase enzyme, hydrogen peroxide (H_2O_2) will be converted into hydroxyl radicals via the Fenton reaction. Hydroxyl radical ($OH*$) is a highly reactive molecule and can react with proteins, nucleic acids, lipids, and other molecules in the body [44,45].

$$H_2O_2 + e^- + H^+ \longrightarrow OH^* + H_2O \; Hydroxyl\ radical \quad (5)$$

More oxygen in the blood that is reduced to ROS will cause the amount of oxygen carried by hemoglobin to decrease. The mechanism that causes a hemoglobin decrease in the blood in diabetes mellitus can be seen in **Figure 6**.

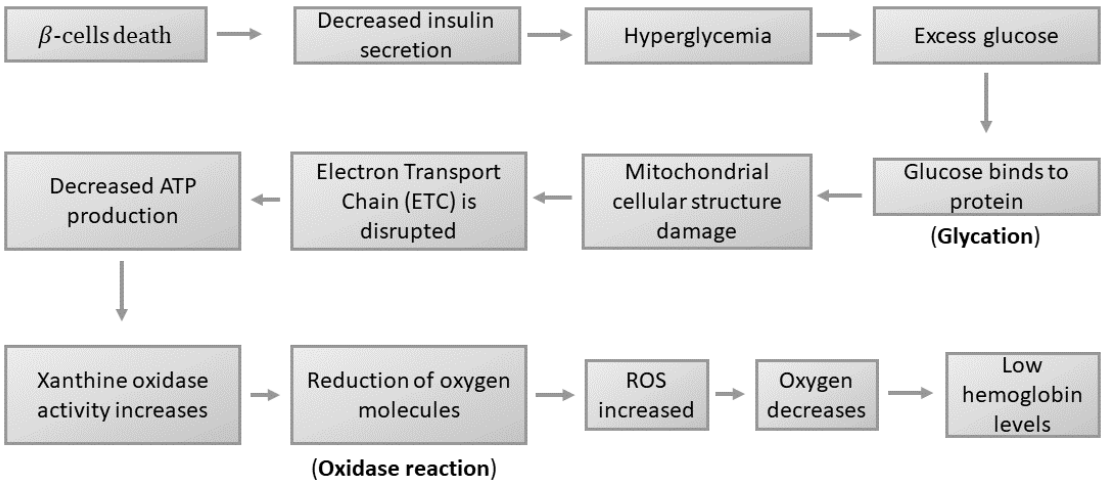

Figure 6 Mechanism of decreasing hemoglobin levels when diabetes mellitus occurs.

Diabetes mellitus can increase the production of superoxide anion radicals ($*O_2^-$), hydroxyl radicals ($OH*$), and hydrogen peroxide molecules (H_2O_2). A series of glycolysis processes disrupted by oxidative stress leads to changes in the structure of red blood cells (RBCs) and hemoglobin's structure. Free radicals will oxidize the heme in hemoglobin, subsequently altering its molecular structure so that it can no longer effectively bind and transport oxygen [8,9]. The structural damage to hemoglobin decreases the oxygen transport cycle and leads to an overall decrease in hemoglobin levels. The greater the number of free radicals, the more hemoglobin degradation will occur continuously [7]. Another pathway free radicals can lower hemoglobin levels is oxidizing the Fe^{2+} ions in hemoglobin to Fe^{3+}. Superoxide anion radicals ($*O_2^-$) draw electrons from Fe^{2+} ions to Fe^{3+}, resulting in methemoglobin (MetHb). Methemoglobin cannot bind oxygen efficiently because Fe^{3+} lacks sufficient affinity to bind oxygen molecules. This series of processes explains how diabetes affects the decrease in hemoglobin levels [5].

Diabetes mellitus will be more severe when hydroxyl radicals appear because hydroxyl radicals can interact with almost all biological substrates. Hydroxyl radicals ($OH*$) will interact with phospholipid and glycolipid components of cell membranes [46]. Cell membrane phospholipids and glycolipids will tend to experience degradation when interacting with free radicals because they have many double bonds. The degradation process of unsaturated fatty acids by free radicals is called the lipid peroxidation reaction. Lipid peroxidation is initiated by releasing one hydrogen atom from the methylene group (C-H). Hydrogen atom release from the methylene group directly adjacent to the double bond (C=C) produces a radical unsaturated fatty acid (RH*) [24,47,48].

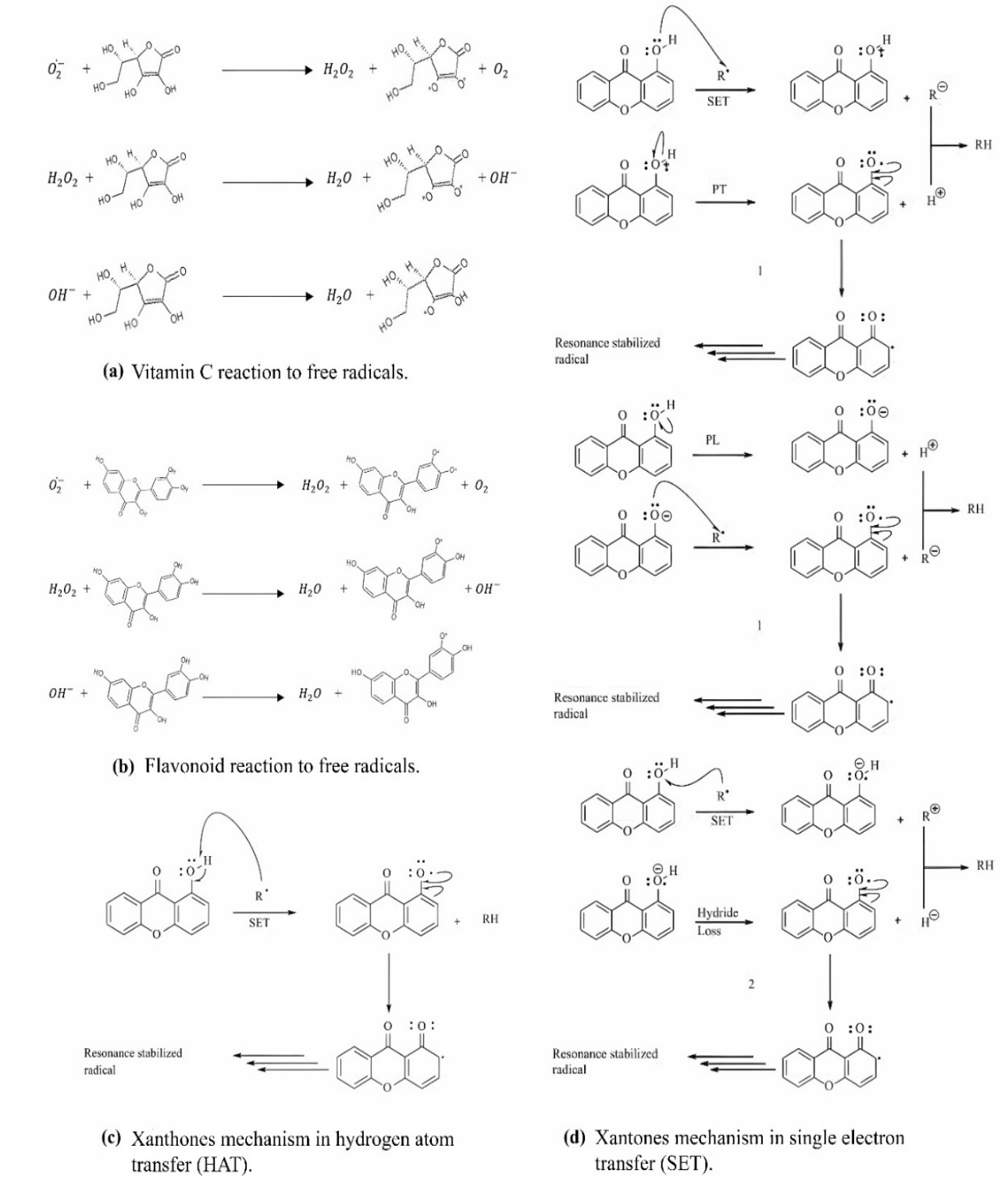

Figure 7 Mechanism of vitamin C, flavonoids, and xanthones when reacting with free radicals [49,50].

Radical unsaturated fatty acids (RH*) then undergo rearrangement by forming conjugated dienes. Radical unsaturated fatty acids (RH*) that have undergone rearrangement will react with oxygen to produce peroxide radicals (ROO*). This event will occur continuously in the body and produce other new fatty acid radicals. Peroxide radicals (ROO*) can also attract hydrogen ions to produce fatty acid hydrogen peroxide (ROOH). Fatty acid hydrogen peroxide (ROOH) is a primary product of lipid peroxidation reactions that is cytotoxic and unstable. When fatty acid peroxide (ROOH) reacts with metal compounds, fatty acid peroxide (ROOH) is broken down into shorter carbon chain compounds and produces the final product

in the form of an aldehyde compound, which is also toxic to the body [51,52].

Our findings show that Mangosteen peel extract (*Garcinia mangostana*) has been proven to suppress free radicals due to its high antioxidant content. The content of vitamin C, flavonoids, and xanthones can react effectively to reduce free radicals number in the body [17,18]. The mechanism of vitamin C, flavonoids, and xanthones is shown in **Figure 7**. The mechanism of vitamin C, flavonoids, and xanthone when reacting with free radicals is by counteracting the chain reaction process from superoxide anions ($*O_2^-$) to hydroxyl radicals ($OH*$). Xantone has a strong phenolic group so it can prevent lipid peroxidation reactions from occurring [17,20,53]. Xanthones react with free radicals in 2 ways, namely single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms [50].

Conclusions

Diabetes mellitus condition is correlated with increased free radicals' relative levels and decreased hemoglobin levels in the blood. Types of free radicals found are superoxide anions ($*O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH*). Mangosteen peel extract (*Garcinia mangostana*) has been proven effective in increasing hemoglobin levels in pre-diabetes group by 9.4 % (PO-1), 13.7 % (PO-2), 24 % (PO-3), 1.4 % (PO-4) and 1.5 % (PO-5) and diabetes mellitus group was 14.3 % (DO-1), 36.5 % (DO-2), 61.5 % (DO-3), 43.7 % (DO-4) and 22.1 % (DO-5). Most effective dose of mangosteen peel extract (*Garcinia mangostana*) is 2.86 mg/mL. The mechanism of vitamin C, flavonoids, and xanthone when reacting with free radicals is by counteracting the chain reaction process from superoxide anions ($*O_2^-$) to hydroxyl radicals ($OH*$). Xanthones in mangosteen peel have phenolic groups that can prevent lipid peroxidation reactions from occurring. Xanthones react with free radicals in 2 ways, namely single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms.

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Ethics

Ethics Committee Approval: Brawijaya University, Indonesia Local Ethics Committee of the host approved the research (ethics approval number: 128-KEP-UB 2023 date: 21.08.2023).

References

[1] MA Darenskaya, LI Kolesnikova and SI Kolesnikov. Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bulletin of Experimental Biology and Medicine* 2021; **171(2)**, 179-189.

[2] K Jakubczyk, K Dec, J Kałduńska, D Kawczuga, J Kochman and K Janda. Reactive oxygen species-sources, functions, oxidative damage. *Polski merkuriusz lekarski: Organ Polskiego Towarzystwa Lekarskiego* 2020; **48(284)**, 124-127.

[3] S Perrone, E Laschi and G Buonocore. Biomarkers of oxidative stress in the fetus and in the newborn. *Free Radical Biology and Medicine* 2019; **142**, 23-31.

[4] BT Bekele, TG Demie and F Worku. Health-related quality-of-Life and associated factors among children and adolescents with type 1 diabetes mellitus: A cross-sectional study. *Pediatric Health, Medicine and Therapeutics* 2022; **13**, 243-256.

[5] H Yaribeygi, SL Atkin and A Sahebkar. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. *Journal of Cellular Physiology* 2019; **234(2)**, 1300-1312.

[6] WN Mratbaevna and S Farrux. The structure of the heart and its physiology in regular athletes. *ACADEMICIA: An International Multidisciplinary Research Journal* 2022; **13(8)**, 41-46.

[7] R Kohan, A Collin, S Guizzardi, N Tolosa de Talamoni and G Picotto. Reactive oxygen species in cancer: A paradox between pro-and anti-tumour activities. *Cancer Chemotherapy and Pharmacology* 2020; **86(1)**, 1-13.

[8] P González, P Lozano, G Ros and F Solano. Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic

interconnections. *International Journal of Molecular Sciences* 2023; **24(11)**, 9352.

[9] J Liang, Y Gao, Z Feng, B Zhang, Z Na and D Li. Reactive oxygen species and ovarian diseases: Antioxidant strategies. *Redox Biology* 2023; **62**, 102659.

[10] A Dobi, S Rosanaly, A Devin, P Baret, O Meilhac, GJ Harry, CL D'Hellencourt and P Rondeau. Advanced glycation end-products disrupt brain microvascular endothelial cell barrier: The role of mitochondria and oxidative stress. *Microvascular Research* 2021; **133**, 104098.

[11] M Faghir-Ganji, N Abdolmohammadi, M Nikbina, A Amanollahi, A Ansari-Moghaddam, R Khezri and H Baradaran. Prevalence of anemia in patients with diabetes mellitus: A systematic review and meta-analysis. *Biomedical and Environmental Sciences* 2024; **37(1)**, 96-107.

[12] AS Kumar, AG Maiya, BA Shastry, K Vaishali, N Ravishankar, A Hazari, S Gundmi and R Jadhav. Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis. *Annals of Physical and Rehabilitation Medicine* 2019; **62**, 98-103.

[13] C Mathieu, PJ Martens and R Vangoitsenhoven. One hundred years of insulin therapy. *Nature Reviews Endocrinology* 2021; **17(12)**, 15-25.

[14] MC Nauman and JJ Johnson. The purple mangosteen (*Garcinia mangostana*): Defining the anticancer potential of selected xanthones. *Pharmacological Research* 2022; **175**, 106032.

[15] A Nasrullah, B Saad, AH Bhat, AS Khan, M Danish, MH Isa and A Naeem. Mangosteen peel waste as a sustainable precursor for high surface area mesoporous activated carbon: Characterization and application for methylene blue removal. *Journal of Cleaner Production* 2019; **211**, 1190-1200.

[16] WM Aizat, FH Ahmad-Hashim and SNS Jaafar. Valorization of mangosteen, "The Queen of Fruits," and new advances in postharvest and in food and engineering applications: A review. *Journal of Advanced Research* 2019; **20**, 61-70.

[17] RRNS Pushpa, KS Babulal, R Kumaran, G Shoba and MD Balakumaran. *In silico* molecular interaction analysis and pharmacokinetic profiling of flavonoids from *Catharanthus roseus* (flower) against TXNIP protein. *Trends in Sciences* 2023; **20(1)**, 6394.

[18] LS Kalick, HA Khan, E Maung, Y Baez, AN Atkinson, CE Wallace, F Day, BE Delgadillo, A Mondal, R Watanapokasin, SM Barbalho and A Bishayee. Mangosteen for malignancy prevention and intervention: Current evidence, molecular mechanisms, and future perspectives. *Pharmacological Research* 2023; **188**, 106-630.

[19] M Masullo, A Cerulli, C Cannavacciuolo, H Kılınç, C Pizza and S Piacente. *Garcinia mangostana* L. fruits and derived food supplements: Identification and quantitative determination of bioactive xanthones by NMR analysis. *Journal of Pharmaceutical and Biomedical Analysis* 2022; **218**, 114-335.

[20] M Patrick, WNWM Zohdi, S Abd Muid and E Omar. Alpha (α)-mangostin (Xanthone of *Garcinia mangostana* L.): Augmenting macrophages activity for an effective diabetic wound healing. *Trends in Sciences* 2024; **21(10)**, 8254.

[21] KN Lesa, N Ahmad, Y Mayangsari, MN Cahyanto and WD Saputra. Anti-diabetic effect of okara noodles on streptozotocin-nicotinamide induced diabetic rats. *Trends in Sciences* 2024; **21(5)**, 7428.

[22] R Cecchini and AL Cecchini. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. *Medical Hypotheses* 2020; **143**, 101102.

[23] JH Kim, YB Yu and JH Choi. Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *Journal of Hazardous Materials* 2021; **413**, 125423.

[24] S Sakuljaitrong, N Buddhakala, M Buddhakala, S Asawawibul, S Buatone, Y Tongaram and C Talubmook. Phytochemicals, cytotoxicity, inhibitory effect on nitric oxide production and alpha-glucosidase activity, and DPPH scavenging activity of leaf extract from *Gymnema inodorum* (Lour.) Decne. *Trends in Sciences* 2023; **20**, 6548.

[25] Z Chen, R Tian, Z She, J Cai and H Li. Role of oxidative stress in the pathogenesis of

nonalcoholic fatty liver disease. *Free Radical Biology and Medicine* 2020; **152**, 116-141.

[26] J Yang, D Zeng, M Hassan, Z Ma, L Dong, Y Xie and Y He. Efficient degradation of Bisphenol A by dielectric barrier discharge non-thermal plasma: Performance, degradation pathways and mechanistic consideration. *Chemosphere* 2022; **286**, 131627.

[27] TJ Costa, PR Barros, C Arce, JD Santos, JD Silva-Neto, G Egea, AP Dantas, RC Tostes and F Jiménez-Altayó. The homeostatic role of hydrogen peroxide, superoxide anion and nitric oxide in the vasculature. *Free Radical Biology and Medicine* 2021; **162**, 615-635.

[28] Y Ahmed, J Zhong, Z Yuan and J Guo. Roles of reactive oxygen species in antibiotic resistant bacteria inactivation and micropollutant degradation in Fenton and photo-Fenton processes. *Journal of Hazardous Materials* 2022; **430**, 128408.

[29] N Maheshwari, N Qasim, R Anjum and R Mahmood. Fluoride enhances generation of reactive oxygen and nitrogen species, oxidizes hemoglobin, lowers antioxidant power and inhibits transmembrane electron transport in isolated human red blood cells. *Ecotoxicology and Environmental Safety* 2021; **208**, 111611.

[30] JW Weisel and RI Litvinov. Red blood cells: The forgotten player in hemostasis and thrombosis. *Journal of Thrombosis and Haemostasis* 2019; **17(2)**, 271-282.

[31] SH Son, JM Oh, P Gangadaran, HD Ji, HW Lee, RL Rajendran, SH Baek, A Gopal, S Kalimuthu, SY Jeong, SW Lee, J Lee and BC Ahn. White blood cell labeling with Technetium-99m (99mTc) using red blood cell extracellular vesicles-mimetics. *Blood Cells, Molecules, and Diseases* 2020; **80**, 102375.

[32] T Pesen, M Haydaroglu, S Capar, U Parlatan and MB Unlu. Comparison of the human's and camel's red blood cell deformability by optical tweezers and Raman spectroscopy. *Biochemistry and Biophysics Reports* 2023; **35**, 101490.

[33] S Lin, Y Shen, X Huang, S Lin and X Shi. *Schefflera arboricola* tablets relieve diabetic neuropathic pain: Involvement of oxidative stress and inflammation. *Pharmacognosy Magazine* 2023; **19(4)**, 906-916.

[34] K Zhao, E Seeliger, T Niendorf and Z Liu. Noninvasive assessment of diabetic kidney disease with MRI: Hype or hope? *Journal of Magnetic Resonance Imaging* 2024; **59(5)**, 1494-1513.

[35] SH Azeez, SN Jafar, Z Aziziaram, L Fang, AH Mawlood and MF Ercisli. Insulin-producing cells from bone marrow stem cells versus injectable insulin for the treatment of rats with type I diabetes. *Cellular, Molecular and Biomedical Reports* 2021; **1(1)**, 42-51.

[36] R Amelia, FM Said, F Yasmin and H Harun. The potential of West Sumatran Dadiah as the novel to alleviate hyperglycemia, hypercholesterolemia, and reducing NF-kB expression in nephropathy diabetes rat model. *Trends in Sciences* 2023; **20(11)**, 6812.

[37] SJ Sharkey, PA Harnedy-Rothwell, PJ Allsopp, LE Hollywood, RJ FitzGerald and FPM O'Harte. A narrative review of the anti-hyperglycemic and satiating effects of fish protein hydrolysates and their bioactive peptides. *Molecular Nutrition & Food Research* 2020; **64(21)**, 2000403.

[38] H Ki, H Jang, J Oh, GR Han, H Lee, S Kim and MG Kim. Simultaneous detection of serum glucose and glycated albumin on a paper-based sensor for acute hyperglycemia and diabetes mellitus. *Analytical Chemistry* 2020; **92(17)**, 11530-11534.

[39] F Akhter, D Chen, A Akhter, SF Yan and SSD Yan. Age-dependent accumulation of dicarbonyls and advanced glycation endproducts (AGEs) associates with mitochondrial stress. *Free Radical Biology and Medicine* 2021; **164**, 429-438.

[40] SM Graves, Z Xie, KA Stout, E Zampese, LF Burbulla, JC Shih, J Kondapalli, T Patriarchi, LTian, L Brichta, P Greengard, D Krainc, PT Schumacker and DJ Surmeier. Dopamine metabolism by a monoamine oxidase mitochondrial shuttle activates the electron transport chain. *Nature Neuroscience* 2020; **23**, 15-20.

[41] H Park, JY Lee, W Lim and G Song. Assessment of the *in vivo* genotoxicity of pendimethalin via mitochondrial bioenergetics and transcriptional

profiles during embryogenesis in zebrafish: Implication of electron transport chain activity and developmental defects. *Journal of Hazardous Materials* 2021; **411**, 125153.

[42] N Zeng, G Zhang, X Hu, J Pan and D Gong. Mechanism of fisetin suppressing superoxide anion and xanthine oxidase activity. *Journal of Functional Foods* 2019; **58**, 1-10.

[43] C Liu, W Fan, WX Cheng, Y Gu, Y Chen, W Zhou, XF Yu, M Chen, M Zhu, K Fan and QY Luo. Red emissive carbon dot superoxide dismutase nanozyme for bioimaging and ameliorating acute lung injury. *Advanced Functional Materials* 2023; **33(19)**, 2213856.

[44] Y Liu, Y Zhao and J Wang. Fenton/Fenton-like processes with in-situ production of hydrogen peroxide/hydroxyl radical for degradation of emerging contaminants: Advances and prospects. *Journal of Hazardous Materials* 2021; **404**, 124191.

[45] H Zeng, G Zhang, Q Ji, H Liu, X Hua, H Xia, M Sillanpää and J Qu. pH-independent production of hydroxyl radical from atomic H*-mediated electrocatalytic H2O2 reduction: A green Fenton process without byproducts. *Environmental Science & Technology* 2020; **54(22)**, 14725-14731.

[46] A Lin, Y Gorbanev, JD Backer, JV Loenhout, WV Boxem, F Lemière, P Cos, S Dewilde, E Smits and A Bogaerts. Non-thermal plasma as a unique delivery system of short-lived reactive oxygen and nitrogen species for immunogenic cell death in melanoma cells. *Advanced Science* 2019; **6(6)**, 1802062.

[47] VG Sprygin, NF Kushnerova and SE Fomenko. Effect of a lipid complex from the Marine Red Alga *Ahnfeltia tobuchiensis* on the metabolic responses of the liver under conditions of experimental toxic hepatitis. *Biology Bulletin* 2021; **48(S3)**, S10-S18.

[48] LFT Novaes, JSK Ho, K Mao, K Liu, M Tanwar, M Neurock, E Villemure, JA Terrett and S Lin. Exploring electrochemical C(sp^3) - H oxidation for the late-stage methylation of complex molecules. *Journal of the American Chemical Society* 2022; **144(3)**, 1187-1197.

[49] M Faisal, UP Juswono and DR Santoso. The effect of extract guava, bitter melon, broccoli, and shallots on liver damage due to indoor pollutant exposure. *Journal of Physics: Conference Series* 2021; **1825**, 012097.

[50] Z Salman, J Yu-Qing, L Bin, P Cai-Yun, CM Iqbal, R Atta-ur and W Wei. Antioxidant nature adds further therapeutic value: An updated review on natural xanthones and their glycosides. *Digital Chinese Medicine* 2019; **2(3)**, 166-192.

[51] MA Fazal, S Rubaiee, A Al-Zahrani and S Ghazali. Biodiesel degradation mechanism upon exposure of metal surfaces: A study on biodiesel sustainability. *Fuel* 2022; **310**, 122341.

[52] X Zhang, N Li, Z Wei, B Dai, H Lin and S Han. Enhanced the effects on improving the cold flow properties and oxidative stability of diesel-biodiesel blends by grafting antioxidant on PMA type pour point depressant. *Fuel Processing Technology* 2022; **238**, 107483.

[53] Y Lu, T Guan, S Wang, C Zhou, M Wang, X Wang, K Zhang, X Han, J Lin, Q Tang, C Wang and W Zhou. Novel xanthone antibacterials: Semi-synthesis, biological evaluation, and the action mechanisms. *Bioorganic & Medicinal Chemistry* 2023; **83**, 117232.