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# The Effect Of Papaya Leaf Extract (Carica Papaya L) On Methemoglobin Percentage And The Number Of Erythrocyte Cells In Wistar Strain White Male Rats Induced With Sodium Nitrite

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## ABSTRACT

Sodium nitrite is widely used in the food industry. Overdosecan cause poisoning, which causes the oxidation of the iron component in the hemoglobin of erythrocytes to methemoglobin, thereby damaging cell membranes and hemolytic. Papaya leaves contain antioxidants that can inhibit the oxidation of iron components in hemoglobin. To determine the effect of papaya leaf extract on methemoglobin levels and the number of erythrocytes in white male Wistar rats strain induced with sodium nitrite. Methodology : True experimental research design using post test only control group design. The object of this study was male white rats induced intraperitoneally with sodium nitrite. The study was divided into a negative control group, a positive control group, a treatment group with a dose of papaya leaf extract 0.7 ml/200 g BW, 1.4 ml/200 g BW, and 2.8 ml/200 g BW). BB). Kruskal-Wallis test, methemoglobin levels sig. 0.001 ($p<0.05$). One way annova test, erythrocyte cell count sig. 0.021 ($p<0.05$). Post Hoc test there was a significant difference between the negative control group and the positive control group on all variables, not different from the second group for methemoglobin levels, not significantly different from the three treatment groups for the number of erythrocytes. Papaya leaf extract contains antioxidants that can inhibit the formation of methemoglobin, and maintain the number of erythrocytes. Papaya leaf extract can inhibit the formation of methemoglobin, maintain the number of erythrocytes in male Wistar strain rats induced with sodium nitrite.

**Keywords :** Sodium Nitrite, Papaya Leaf Extract, Methemoglobin, Erythrocytes.



## INTRODUCTION

Sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$) are natural chemicals commonly used as food preservatives, especially meat and fish, involving salt, sugar, and/or drying to make food resistant to bacteria that can cause spoilage. Sodium nitrate is often found in vegetables, which in the digestive tract will be converted into nitrite. While sodium nitrite is more widely used as a food preservative.(Alfaro, 2019)

Excessive use of sodium nitrite has been reported to cause poisoning. Katabami et al. (2016) reported a case of severe methemoglobinemia in Japan due to sodium nitrite poisoning in a

28-year-old man who was brought to the emergency department with transient disturbances of consciousness and cyanosis. The man consumed about 15 grams of sodium nitrite an hour before being taken to the hospital (Katabami, Hayakawa, and Gando, 2016). Wang R et al. (2013) reported that three people in a family in China experienced sodium nitrate poisoning after consuming asparagus soup contaminated with sodium nitrite (Wang R et al, 2013). Cruz DM et al. (2018) reported the incidence of sodium nitrite poisoning in a 23-year-old woman after attempting suicide by drinking sodium nitrite. The patient has decreased blood pressure, decreased oxygen saturation, and increased methemoglobin levels in the blood. (Cruz DM. et al., 2018).

Sodium nitrite can cause oxidative stress on the iron component in the hemoglobin molecule of erythrocytes. Methemoglobin is formed when sodium nitrite oxidizes ferrous ($Fe2+$) to ferric ($Fe3+$), known as methemoglobin. Methemoglobinemia occurs when the circulating methemoglobin level is above 1.5%. In healthy humans, erythrocytes are constantly exposed to oxidative stress from natural metabolism. Spontaneous formation of methemoglobin from ferrous hemoglobin is reversed by the protective enzyme systems cytochrome-b5 reductase and nicotinamide adenine dinucleotide phosphate (NADPH) MetHb reductase. This pathway maintains hemoglobin levels below 1.5% in healthy individuals. (Cruz DM et al., 2018). Balkhrisna et al. (2014) reported two cases of poisoning by substances containing sodium nitrite, with complaints of shortness of breath and decreased consciousness, with increased levels of methemoglobin and hemolytic anemia.. (Balkhrisna et al., 2014)

The management of nitrite poisoning is to use methylene blue preparations (Ginimuge, 2010). These drugs have side effectsand are dangerous when given in excess of the dose. Therefore, alternative therapies have been developed using traditional herbal medicines, one of which is carica papaya leaves.

*Carica papaya* leaf contains active components which are responsible for its medicinal activity (Sing et al. (2020). Papaya leaves (Carica papaya I) are rich in antioxidant substances, containing phytochemicals flavonoids 899.53 mg/100 g, alkaloids 1,569.13 mg/100 g, saponins 898.07 mg/100 g, and tannins 310.50 mg /100 g. (Joy U, et al, 2019). The antioxidants in papaya leaf extract have the ability to protect against oxidation reactions from free radicals (Khor BK, et al, 2021)

## MATERIALS AND METHOD

### Study design

This research is a true experimental using a post-test-only control group design. The object of this research is an active male white rat (Rattus novergicus Wistar Strain), 2-3 months old, weighing 150-200 grams. The treatment is by giving papaya leaf extract. There were five treatment groups in this study, namely a normal control group that was only given standard feed and drink, positive control with sodium nitrite induction, and three treatment groups of pure papaya leaf extract 0.7 ml/rat/day, 1.4 ml/day. rats/day, and 2.8 ml/rat/day orally 5 minutes after intraperitoneal injection of sodium nitrite. (Dharmarathna et al., 2013). Estimation of sample size using the Federer formula, each group contains five rats. Data wereanalized using Kruskal-Wallis test, onewayanova test, and post hoc test.

**Sodium nitrite induction**

Sodium nitrite (NaNO2) is administered acutely intraperitoneally 50 mg/kgBW in a single dose. In this study, the body weight of rats was about 200 g, so the sodium nitrite levels for each tail were: 10 mg/200 g of body weight of rats (Gluhcheva*et al.*, 2012a)

**Papaya leaf extract**

Fresh papaya leaves of middle age are washed and cut into pieces; the stems are removed beforehand, weighed as much as 1 kg. Then the leaves are blended without adding water. The leaves that have been blended are filtered to get a pure extract. Volume was measured, and the extract was stored at 4 $^{o}$C. (Dharmarathna et al., 2013). The doses given were 0.7 ml, 1.4 ml, and 2.8 ml / 200 gr rats orally. The extract was administered 5 minutes after sodium nitrite injection. Extract preparation was carried out at the biomedical laboratory of Medical Faculty UMM.

**Parameter measurement**

Mice were killed after 5 hours of acute induction of sodium nitrite and administration of papaya leaf extract with inhalational chloroform anesthetic. Blood was drawn from the rat heart ventricle.

1. Methemoglobin levels were measured visually on filter paper dripping with rat blood, compared with the color using a Shihanamethemoglobincolor card. (Shihana, 2016)
2. The number of erythrocytes was calculated automatically by flow cytometry method using the Sysmex hematoanalyzer

## RESULTS AND DISCUSSION

Sodium nitrite is a preservative widely used for processed meat products and fish and is naturally found in some foods, especially vegetables. Nitrite is also used as a pharmacological drug in certain diseases. Besides that, nitrite can contaminate drinking water, and this substance is widely used in the food industry, which can harm health if not used properly.

The research we did was by induction of an acute intraperitoneal injection of sodium nitrite. Nitrite will cause intoxication of ferrous hemoglobin ion so that it turns into methemoglobin.

**Table1**. Methemoglobin levels

| Group | Methemoglobin (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | x | SD |
| Control (-) | 5 | 5 | 5 | 5 | 5 | 5,00 | 0,00 |
| Control (+) | 45 | 50 | 35 | 45 | - | 43,75 | 6,29 |
| GI | 20 | 20 | 25 | 30 | - | 23,75 | 4,79 |
| GII | 15 | 10 | 10 | 10 | - | 11,25 | 2,50 |
| GIII | 15 | 15 | 10 | 10 | 25 | 15,00 | 6,12 |

Note :

C(-) : negative control

C(+) : positive control

GI : $NaNO_3$ peritoneal injection 10 mg/200 gr BW, Papaya leaft extract 0,7 ml/200 gr BW

GII :$NaNO_3$ peritoneal injection 10 mg/200 gr BW, Papaya leaf extract 1,4 ml/200 gr BW

GIII :$NaNO_3$ peritoneal injection 10 mg/200 gr BW, Papaya leafextract 2,8 ml/200 gr BW

Methemoglobin levels with the Kolmogorov-Smirnov normality test were not normally distributed (p. 0.024), the Levene homogeneity test was homogeneous (p. 0.129). So that the mean difference test uses the Kruskal-Wallis test. There was a significant difference in methemoglobin levels between groups with sig. 0.001 ($p<0.05$). In the Pos Hoc test, it appears that the methemoglobin levels of the negative control group were significantly different from the positive control group (sig. 0.000), significantly different from the GI group (sig. 0.000), not significantly different from the GII group (sig. 0.29), significantly different from GIII group (sig. 0.022). The positive control group was significantly different from the three treatment groups (sig. 0.000). The GI group was significantly different from the GII group (sig. 0.10) but not from the GIII group (sig. 0.072). The GII group was not significantly different from the GIII group (sig. 0.74). In the three treatment groups, there was a decrease in methemoglobin levels. The GII treatment group (pure extract of papaya leaves 1.4 ml /200 gr rats BW) gave the lowest methemoglobin levels among the three treatment groups. In the Pos Hoc test, it was not different from the negative control group. Spearman correlation between papaya leaf dose and methemoglobin level is –0, 47 sig. 0.105 ($p> 0.05$), meaning that there is a non-unidirectional relationship with sufficient strength, but it is less significant.

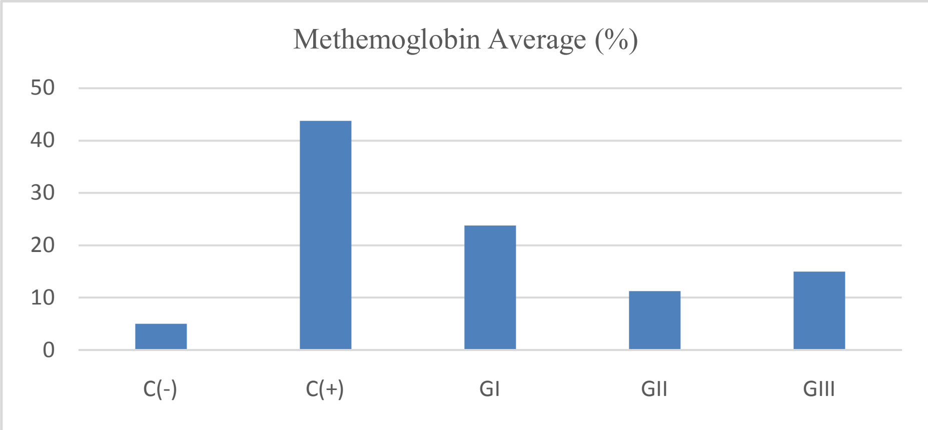


**Figure 1** Histogram of methemoglobin levelaverage

In this study, the mean number of erythrocytes appeared to decrease in the positive control group compared to the negative control group. The mean number of erythrocytes appeared to increase in the treatment group.

**Table 2**. Erythrocyte Cell Count

| Group | Erithrocytes Count ($10^6$/ul) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | x | SD |
| C(-) | 7,70 | 7,87 | 8,50 | 7,76 | 8,30 | 8,02 | 0,35 |
| C(+) | 6,52 | 6,44 | 7,17 | 6,63 | - | 6,69 | 0,32 |
| GI | 7,45 | 6,93 | 7,70 | 7,08 | - | 7,29 | 0,34 |
| GII | 7,68 | 6,93 | 6,96 | 8,01 | - | 7,39 | 0,53 |
| GIII | 7,85 | 7,77 | 8,24 | 6,61 | 9,30 | 7,95 | 0,96 |

The number of erythrocytes has a normal distribution of data (sig. 0.40) and is homogeneous (sig. 0.34). One way ANOVA test there are significant differences between the treatment groups sig. 0.021(<0.05). A histogram of the mean number of erythrocytes showed a decrease in the negative control group and an increase in the treatment group. In the post hoc test, the number of erythrocytes in the negative control group was significantly different from the positive control group (sig. 0.024), not significantly different from the three treatment groups (sig > 0.05). The positive control group was not significantly different from the GI group (sig. 0.605), not significantly different from the GII group (sig. 0.45), but significantly different from the GIII group (sig. 0.035). In the three treatment groups, there was no significant difference ($p > 0.05$). In this study, administration of papaya leaf extract at a dose of 2.8 ml/200 g body weight rats seemed to maintain the number of erythrocytes after acute induction of sodium nitrite. Spearman correlation between papaya leaf dose and erythrocyte cell count was 0.414 sig. 0.16 ($p > 0.05$), means that there is a unidirectional relationship with sufficient strength, but it is less significant.

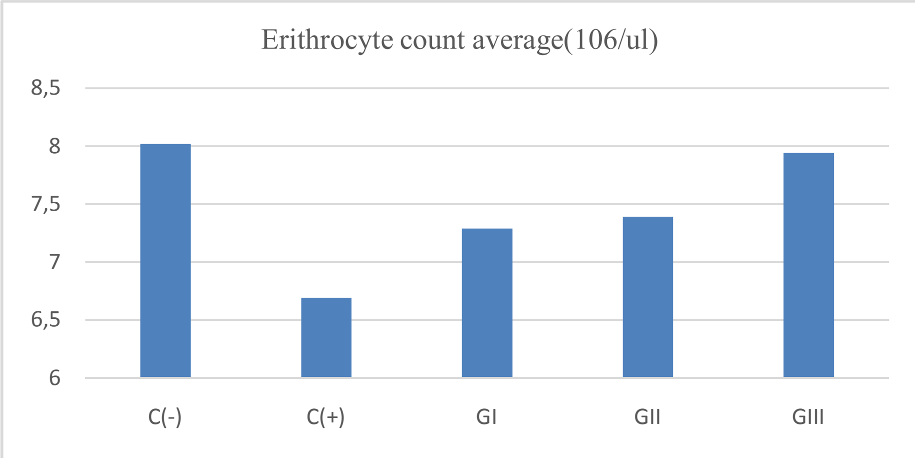


**Figure 2.** HistogramErythrocyte Cell Count Average

In this study, the effect of giving nitrite to rats was similar to that of Gluhcheva et al. (2012). This experimental study was conducted on rats by injecting a single dose of sodium nitrite 50 mg/Kg BW intraperitoneally. At the 5th hour after injection, there was a significant decrease in erythrocytes compared to the negative control group (normal group). The chemical reaction of sodium nitrite causes increased heme toxicity in erythrocyte hemoglobin. Nitrite causes the formation of free radicals, which stimulate the oxidation of ferrous ions in oxyhemoglobin to form methemoglobin, reactive oxidant stress (ROS). The nitrite ion and its metabolites and the resulting lipid peroxidation products react with sulfhydryl groups in the lipid bilayer and protein components of the erythrocyte membrane and cause structural changes. Nitrite causes an influx of Ca2+ ions in blood cells that activate phospholipase, which will increase the proportion of phospholipids in the membrane so that the structure becomes rigid. (Gluhcheva et al., 2012; Titov VY, PetrenkoYM, 2005). Changes like the fixed erythrocyte cell membrane cause erythrocyte cells to easily undergo lysis. The lysis of erythrocyte cells causes a decrease in the number of erythrocytes.

The study is also in accordance with that conducted by Hamidah et al (2017), by giving induction of sodium nitrite 0.4 ml/20 g of mice orally per day and treatment of papaya leaf juice diet for 14 days after anemia occurs. In this study, papaya leaf juice diet with a concentration of 75% gave a significant increase in the number of erythrocytes.

In this study, the methemoglobin level in the negative control group was not different from the GII group, but the correlation of papaya extract dose with the decrease in methemoglobin level was less significant. It is possible that there are endogenous factors in mice that cause physiological differences in mice. As is known in the body there is a natural defense mechanism, in which the spontaneous formation of methemoglobin from ferrous hemoglobin will be reversed by the protective enzyme system cytochrome-b5 reductase and nicotinamide adenine dinucleotide phosphate (NADPH) MetHb reductase. This pathway maintains hemoglobin levels below 1.5% in healthy people. (Cruz DM*et al.*, 2018). In this study, papaya leaf extract used papaya leaves of middle age. However, according to Nisa et al (2019), the papaya leaf extract with the highest antioxidant activity is the mature level.

The antioxidants in papaya leaf extract have the ability to protect against oxidation reactions from free radicals (Khor BK, et al, 2021). Study by Banu KS (2018) , was carried out to analyze the antioxidant activity of the plant Carica papaya leaf extract (Wild Species). The scavenging activity of the plant extract through the annihilation of the DPPH radicals was investigated.The Carica papaya leaf extract (Wild Species) is found to have the antioxidant activity. The antioxidant activity of the Carica papaya leaf extract (Wild Species) increases as the concentration increases. Higher concentration of carica leaf extract shows higher antioxidant activity. Extract Carica papaya can counteract pro-oxidants via a number of signaling pathways that either promote the expression of antioxidant enzymes or reduce ROS production. These signaling pathways activate the antioxidant defense mechanisms that protect the body against both intrinsic and extrinsic oxidative stress (Kong YR et al, 2021)

In this study, the number of erythrocyte cells in the three treatment groups did not appear to be significantly different from the negative control group, but the correlation between increasing the dose of extract and the number of erythrocytes was less significant. Papaya leaf extract contains antioxidant phytochemicals flavonoids 899.53 mg/100g, alkaloids 1569.13 mg/100g, saponins 898.07 mg/100g, and tannins 310.50 mg/100g. (Joy Ugo et al, 2019). Papaya leaf extract was investigated to have antioxidant properties that can reduce $Fe3+$ to $Fe2+$. (Foyzun and Aktar, 2017). There is a study measuring the iron-reducing strength of papaya extract, by assessing the antioxidant activity. The reducing power of $Fe3+$ from crude methanolic extract (CME) and crude ethyl acetate extract (CEE) of papaya leaves increased with the concentration of the extracts. Both kinds of extracts increased their reducing ability with moderate yield compared to standard ascorbic acid. CME seems to have the highest capability compared to CEE. (Foyzun and Aktar, 2017). In our study, the papaya leaf extract given was pure extract without solvents, the possibility of the antioxidant active substance being lower than papaya leaf extract using CEE.

Gheith I and Mahmoudy (2019) have done research, with result showing that the daily administration of *CPLE* significantly affected both the erythrocyte and leukocyte parameters compared with the control-group ($P$>0.05). The effect was toward the physiological direction and its rate was directly proportional to the dose. Moreover, it was almost as that of the standard folate/ascorbate anti-anemic mixture used in the study. *CPLE* at small and large doses, significantly restored RBCs count, HGB, and PCV values that were deteriorated by successive administration of the nephrotoxin and gentamicin. While Asadullah et al (2016), researched that the papaya plant leaves extract are posses hematopoietic potential, as their use in healthy rabbits significant increase the hemoglobin level and thus it could be use pharmacologically for the treatment of anemia.Dharmarathna SLCA, et al (2013), innvestigate the potential role of fresh *Carica papaya* (*C. papaya*) leaf extract on haematological and biochemical parameters and toxicological changes in a murine model. They reported that Fresh *C. papaya* leaf extract significantly increased the platelet and RBC counts in the test group as compared to controls.In another research, dried *C. papaya* leaves were extracted using the soxhlet extraction method with 5 different solvents to give five different fractions, namely, hexane, chloroform, ethyl acetate, butanol, and water. The research examined the crude extract and the various leaf extract fractions of *C. papaya* L. (Caricaceae) for possible *in vitro* antisickling activities on $Hb^{ss}$ red blood cells obtained from noncrisis state sickle cell patients involving the use of positive (phydroxybenzoic acid 5 $\mu$g/mL) and negative (normal saline) controls for the antisickling experiments. Pretreatment of SS cell suspensions with *C. papaya* leaf extract and fractions all inhibited formation of sickle cells under severe hypoxia at varying degrees, with only 0–5% sickle cells in the crude extract at 60 min compared with untreated SS cell suspensions which had over 80% sickle cells. Analysis of two different concentrations of *C. papaya* crude extract (10 and 5 mg/mL) showed the 10 mg/mL extract as the concentration with highest antisickling effect. Butanol extract showed the highest antisickling activity at 10 mg/mL concentration, while the ethyl acetate extract had the highest antisickling activity at 5 mg/mL concentration.These results further indicate the possibility of *C. papaya* leaf extract as potential phytotherapy for sickle cell anemia (Imaga NA, 2013). The aqueousextract of Carica papaya leaf also has anti-sickling effect which may be related to its direct action onHaemoglobinS and also its membrane stabilizing ability. This extract therefore may be a good candidate in Sickle Cell Disease therapy (Naiho AO et al, 2015).The potential of aqueous extracts of Cajanus cajan leaf and seed, Zanthoxylum zanthoxyloides leaf, and Carica papaya leaf in sickle cell disease management was investigated in vitro using freshly prepared 2% sodium metabisulfite for sickling induction. The results indicated that the percentage of sickled cells, which was initially 91.6% in the control, was reduced to 29.3%, 41.7%, 32.8%, 38.2%, 47.6%, in the presence of hydroxyurea, C. cajan seed, C. cajan leaf, Z. zanthoxyloides leaf, and C. papaya leaf extracts, respectively, where the rate of polymerization inhibition was 6.5, 5.9, 8.0, 6.6, and 6.0 ($\times 10^{-2}$ ) accordingly. It was also found that the RBC resistance to hemolysis was increased in the presence of the tested agents as indicated by the reduction of the percentage of hemolyzed cells from 100% to 0% (Nurain IO et al., 2016).

The safettstudy evaluated the potential toxicity of the hydroalcoholic extracts of Aloe vera leaves, Carica papaya leaves or seeds, and Mimosa pudica leaves after acute and sub-chronic administration in chicks.The results showed that the therapeutic use of the hydroalcoholic extracts of Aloe vera leaves, Carica papaya leaves or seeds and Mimosa pudica leaves had very low toxicity in oral acute high dose administration and no toxicity in oral sub-chronic low dose administration and indicate that the plants could be considered safe for oral medication in chicks (Nghonjuyi et al, 2015). Ismail et al (2014) also have investigated the safety of Carica papaya leaf extract, with the results that there were no significant differences observed in hematology parameters between treatment and control groups; however significant differences were seen in biochemistry values, for example, LDH, creatinine, total protein, and albumin. However, these changes were not associated with histopathological changes. In conclusion, the results suggested that daily oral administration of rats with C. papaya leaf extract for 13 weeks at a dose up to fourteen times the levels employed in traditional medicine practice did not cause any significant toxic effect.

## CONCLUSION

Papaya leaf extract can prevent the increase in methemoglobin levels, and maintain the number of erythrocyte cells in male Wistar strain rats induced by peritoneal injection of sodium nitrite.We recommend further research using other solvent of papaya leaf extract in lowering methemoglobin levels.

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