

## Ameliorative effect of 50% ethanol extract of moringa leaves (*Moringa oleifera* Lam.) on lead-induced oxidative stress in the liver of male wistar rat model



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### ABSTRACT

**Purpose:** This study aimed to examine the ameliorative effect of 50% ethanol extract of Moringa leaves on lead-induced oxidative stress in the liver of male Wistar rat model.

**Experimental Animal and methods:** In this study, adult male Wistar rats were divided into 4 groups, consists of one control group (Kn) and three experimental groups (P1, P2 and P3). All group received Pb-acetate 750 mg/kgBW/day for 7 days. After that, control groups received 1 ml aqua for 14 days, and 3 experimental groups received 1 ml volume of 250, 500 and 1.000 mg/kgBW/day of 50% ethanol extract of moringa leaves orally for 14 days, respectively. Methods measured liver level of GSH, GPx, SOD, CAT and MDA describes by Hernayanti and Simanjuntak (2018) and Ratnaningtyas et al (2022). GSH was measured by a method described by El Shater et al (2016). Data were analyzed with ANOVA and Tukey HSD post hoc test.

**Results:** Study results demonstrated that there was significant elevation of liver level of GSH, GPx, SOD, and CAT ( $p < 0,05$ ), and significant decreased of MDA levels ( $p < 0,05$ ) in all experimental groups. Significant amelioration of oxidative stress ( $p < 0,05$ ) were found in groups received 250, 500 and 1.000 mg/kgBW/day orally for 14 days.

**Conclusion:** In conclusion, 50% ethanol extract of moringa leaves doses 250, 500 and 1.000 mg/kgBW/day orally for 14 days ameliorates lead-induced oxidative stress in rat liver. The most effective dose was 1.000 mg/kgBB/day orally for 14 days.

**Keywords:** ethanol extract; lead; moringa leaves; oxidative stress; rat liver.

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### INTRODUCTION

Lead (Pb) is one of the most dangerous heavy metals.<sup>1</sup> Pb exposure is still a global public health problem in developing and developed countries due to continuous environmental and workplace exposure.<sup>2</sup> Lead enters human body through polluted air, food and drinking water, skin contact, etc. Lead is accumulated in human body, including in liver, kidney, and bone and causes long-term poisoning in the human body. According to the Institute of Health Measurement and Evaluation (IHME), in 2017, chronic lead poisoning results in about 1.06 million people died and 24.4 million people suffered morbidity. In 2016, 63,2% global burden of cognitive disorder were attributable to lead exposure.<sup>3</sup>

The liver is prone to lead toxicity because it is one of the main target organs damaged by Pb exposure. Pb causes adverse effects on liver cells because after

Pb exposure, liver is one of the main organs involved in the storage, biotransformation and detoxification of Pb.<sup>4</sup> Pb absorbed in the body is conjugated in the liver, and then distributed to other organs.<sup>1</sup> Thus, liver cells damage could be happening in the acute stage of Pb poisoning. As such, ameliorating Pb toxicity in the liver plays an important role to minimize adverse effect of Pb exposure in the human body.

The main mechanism of Pb toxicity in the liver is through oxidative stress. In Pb poisoning, the formation of free radicals exceeds the ability of the body's antioxidant system to detoxify free radicals, resulting in the accumulation of free radicals that cause cell damage.<sup>5</sup> Oxidative stress occurs through enzymatic and non-enzymatic pathways. Pb ions have been shown to be associated with increased ROS formation and can interfere with antioxidant defenses, including antioxidant enzymes

and non-enzymatic antioxidants.<sup>6</sup> In the enzymatic pathway, Pb inactivates glutathione peroxidase (GPx) enzyme.

Other antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), are also inactivated by Pb by means of its capability to take over zinc ions that function as co-factors for COD and CAT enzymes. The sulfhydryl groups of these three enzymes are also targets for Pb. In the non-enzymatic pathway, Pb that has strong electron-sharing properties, helps the formation of covalent bonds. Covalent bonds are formed from lead groups and sulfhydryl groups in antioxidant enzymes, which in turn make antioxidant enzymes inactive. Pb also binds to the sulfhydryl group of reduced glutathione (GSH) and inactivates it.<sup>5</sup> Decreased activity of antioxidant enzymes and GSH causes lipid membranes cell damage through lipid peroxidation indicated by increased

malondialdehyde (MDA) levels.<sup>1</sup>

Dimercaptosuccinic acid (DMSA) and calcium disodium ethylenediaminetetraacetate (CaNa<sub>2</sub>-EDTA) are the most frequently used agents to chelate Pb and excreted it from the human body. However, in addition to Pb chelating, DMSA and CaNa<sub>2</sub>-EDTA also excreted some essential trace elements, such as calcium, magnesium, copper and zinc, which result is adverse health effects called over complexing syndrome.<sup>3</sup>

Realizing that the main toxic effect of Pb is causing oxidative stress, the alternative treatment in addition to eliminating Pb from the body is to ameliorate oxidative stress induced by Pb exposure. Moringa (*Moringa oleifera* Lam.) leaves, which Indonesian people widely use for nutrition source and traditional remedies for disease treatments, have a good content of polyphenols, especially flavonoids. The three major flavonoids in Moringa leaves extract are myrecetin, quercetin and kaempferol. Flavonoids, which plants synthesize to protect them from microbes' infection, have a benzo-pyrone ring as a general form, and have been demonstrated to prevent from getting disease related to oxidative stress.<sup>7</sup> In Indonesia, traditional remedies commonly use 50% ethanol extract. Zhang et al (2018) also stated that 50% ethanol extract made by maceration method on chokeberry produced the most optimal total phenol.<sup>8</sup> This research aimed to examine the effect of 50% ethanol extract of moringa leaves oral administration on lead-induced oxidative stress in male Wistar rat liver. Ameliorating oxidative stress will protect liver cells from damage caused by oxidative stress triggered by free radicals. As such, liver function in detoxification of Pb poisoning will be more effective.

## MATERIAL AND METHODS

### Ethical clearance

All experimental procedures of this experimental research were endorsed by Medical Research Ethical Committee, Faculty of Medicine, Jenderal Soedirman University, ethical approval number 199/KEPK/IX/2021. There were not any experiment procedural changes in the procedure that has been approved by the committee.

### Research design

This study was true experimental, post-test only with control group design. Research was conducted at Pharmacology Laboratory for experiment process, whereas specimen examination was conducted at Research Laboratory, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia.

### Moringa leaves extraction

Fresh Moringa leaves were collected from a moringa garden in Yogyakarta, Indonesia. Fresh moringa leaves were washed, shaded with cloth and dried under sun shine until dry. Dried moringa leaves were extracted using 50% ethanol in an extractor, and macerated for 24 hours. Maceration was conducted twice. The liquid extract produced from the maceration was processed with a freeze dryer to obtain solid extract. The detailed method was as described in detail by Mahdi et al. (2016). The solid extract was put in the brown bottle and kept in the refrigerator.<sup>9</sup>

### Experiment procedure

Forty-eight local strain adults male Wistar rats were used in this study. The inclusion criteria were weighing 150-200 grams, 2,5-3-month-old, and from the physical examination, the rats were healthy. The rats that sick or died during the research process were excluded from the research process. Before the experimental procedure proceeded, rats were placed in the cage at room temperature (20-24°C) and 40-70% humidity. They were fed with standard commercial rat pellets and tap water. They were acclimated to the laboratory room environment for 7 days before the experiment.

The rats were divided randomly into 4 group, with 12 rats per group. As there is no data about acute Pb exposure to induce oxidative stress from Wistar rats bred in Indonesia, acute Pb exposure dose was determined based on our preliminary study result as modification of research conducted by previous study.<sup>10</sup> Doses of 50% extract ethanol of Moringa leaves were also determined based on previous research.<sup>10</sup> The groups and the treatment of each group were as follows:

Control group (Kn): The rats received Pb-acetate 700 mg/kgBW per day orally

for 7 days, followed by administration of aqua for 14 days

Experiment group 1 (P1): The rats received Pb-acetate 700 mg/kgBW per day orally for 7 days, followed by administration of 250 mg 50% ethanol extract of moringa leaves orally for 14 days.

Experiment group 2 (P2): The rats received Pb-acetate 700 mg/kgBW per day orally for 7 days, followed by administration of 500 mg 50% ethanol extract of moringa leaves orally for 14 days.

Experiment group 3 (P3): The rats received Pb-acetate 700 mg/kgBW per day orally for 7 days, followed by administration of 1.000 mg 50% ethanol extract of moringa leaves orally for 14 days.

Administration of Pb-acetate and 50% ethanol extract of moringa leaves to all rats were conducted in the morning, between 7-8 am, local time.

### Speciment collection

One day after the end of the experiment period, rats' livers were collected after rats were decapitated. The collected livers were washed with 1% PSB solution until they were clean from the blood and put into pot contained BNF 10% solution. The tissue homogenized in 5-10 ml cold buffer and centrifuged at 4.000 rpm for 15 minutes at 40°C. The supernatant was collected and stored at -80°C if the sample is not tested at the same day.

### Laboratory examination

The collected livers were processed for chemical examination and then sent to the research laboratory. Data collected were level of GSH, GPx, SOD, CAT and MDA. The determination of GPx, SOD, CAT and MDA levels were done by spectrophotometry methods as described by Hernayanti and Simanjuntak (2018) and Ratnaningtyas et al. (2022).<sup>11,12</sup> GSH levels were determined by spectrophotometry methods as described by El Shater et al. (2016).<sup>13</sup>

### Statistical analysis

Mean and standard deviation were determined in all groups. One-way ANOVA and Tukey HSD post hoc test

**Table 1.** Laboratory analysis results of GSH, SOD, GPx, CAT and MDA in male Wistar rats liver.

NO.	VARIABLE	GROUP			
		Kn	P1	P2	P3
1	GSH ( $\mu\text{mol}/\text{mg}$ )	$59.23 \pm 1.77$	$62.82 \pm 1.60$	$75.94 \pm 2.96$	$98.05 \pm 4.44$
2	SOD (U/mg)	$48.62 \pm 1.17$	$56.16 \pm 4.21$	$61.62 \pm 3.39$	$68.34 \pm 3.50$
3	GPx (U/mg)	$59.12 \pm 1.59$	$63.88 \pm 5.64$	$71.99 \pm 3.90$	$78.64 \pm 3.53$
4	CAT (U/mg)	$2.69 \pm 0.21$	$3.50 \pm 0.26$	$4.32 \pm 0.69$	$6.03 \pm 0.64$
5	MDA (nmol/gr)	$3.22 \pm 0.12$	$2.76 \pm 0.12$	$2.36 \pm 0.22$	$1.77 \pm 0.20$

**Table 2.** One-way ANOVA Statistic analysis results.

NO.	VARIABLE	One-way ANOVA	
		F value	p value
1	GSH	433.538	0,000
2	SOD	78.164	0,000
3	GPx	57.893	0,000
4	CAT	98.594	0,000
5	MDA	155.509	0,000

**Table 3.** Tukey HSD post hoc test results in the means difference of experimental groups compared to control group.

NO.	VARIABLE	P VALUE		
		GROUP P1	GROUP P2	GROUP P3
1	GSH	0,022	0,000	0,000
2	SOD	0,000	0,000	0,000
3	GPx	0.025	0.000	0.000
4	CAT	0.001	0.000	0.000
5	MDA	0,000	0,000	0,000

were used to analyze the significance of differences in GSH, GPx, SOD, CAT and MDA levels between control group and treatment groups. Analysis results were considered to be significant when the p value < 0,05.

## RESULTS

Based on the data in Table 1, it can be seen that the liver GSH level in the control group (Kn) was lower than that of 3 treatment groups: P1, P2 and P3. The mean GSH levels in the Kn group were  $59.23 \mu\text{mol}/\text{mg}$ , while in the P1, P2 and P3 groups were  $62.82$ ,  $75.94$ , and  $98.05 \mu\text{mol}/\text{mg}$ , respectively. The highest GSH level mean was found in the P3 group. High GSH level means that oxidative stress is ameliorated.

In the hepatic SOD level, the Kn group had lower SOD level than the P1, P2 and P3 groups. The mean SOD level in the Kn group was  $48.62 \text{ U}/\text{mg}$ , whereas in the P1, P2 and P3 groups were  $56.16$ ,  $61.62$ , and  $68.34 \text{ U}/\text{mg}$ , respectively. The group with the highest SOD activity was the P3 group, which received 50% ethanol

extract of moringa leaves. High SOD level indicates that this enzyme is still effectively scavenging free radicals.

GPx level was low in Kn group, and the highest level of GPx was found in Group P3. The mean GPx levels were  $59.12 \text{ U}/\text{mg}$  in Kn Group and  $78.64 \text{ U}/\text{mg}$  in the P3 group. The same pattern was found in CAT level. CAT level result showed the same pattern as GPx level. In Kn group, CAT level means was  $2.69 \text{ U}/\text{mg}$ , whereas in P1, P2 and P3 groups were  $3.50$ ,  $4.32$ , and  $6.03 \text{ U}/\text{mg}$ , respectively.

In contrast to hepatic GSH levels and hepatic SOD level, the mean liver MDA level in the Kn group were higher than those in the P1, P2 and P3 groups. The mean liver MDA levels in the Kn group were  $3.22 \text{ nmol}/\text{gr}$ , while those in the P1, P2 and P3 groups were  $2.76$ ,  $2.36$  and  $1.77 \text{ nmol}/\text{gr}$ , respectively. The lowest liver MDA level was in the P3 group. Lower MDA level indicates that lipid peroxidation could be minimized.

The results of One-way ANOVA could be seen in Table 2. It could be seen that in the One-way ANOVA test, in liver GSH level, the F value = 433.538 and p = 0.000.

In the analysis of SOD level, the value of  $F=78,164$  and  $p=0,000$  and for liver MDA level, the value of  $F = 155.509$  and  $p = 0.000$  were obtained. These results indicate that the ANOVA test could describe all variables and that the analysis results were significant.

Tukey's HSD post hoc test analysis showed that in all variables, significant differences were found between control group (Kn) and all experimental groups P1, P2 and P3 (Table 3). These results indicated that all doses of 50% ethanol extract of Moringa leaves significantly ameliorate stress oxidative in the rat's liver. However, from the p value, 50% ethanol extract of Moringa doses  $1.000 \text{ mg}/\text{kgBB}/\text{day}$  for 14 days, is the most effective dose in ameliorating oxidative stress induced by acute exposure of lead in Wistar rat livers.

## DISCUSSION

Results of this study showed that 50% ethanol extract of Moringa leaves significantly ameliorates oxidative stress in the rat liver. Amelioration was found in both non enzymatic and enzymatic pathways. In non-enzymatic pathway, 50% ethanol extract of Moringa leaves significantly increased GSH level in the rat's liver. In the enzymatic pathway, 50% ethanol extract of Moringa leaves significantly increase SOD activity, GPx and CAT levels. Lipid peroxidase also ameliorated by 50% ethanol extract of Moringa leaves significantly, indicated by lowering MDA level in the rat liver.

Pb is considered one of the most poisonous and widely distributed heavy metals. Previous studies reported that liver is the main deposit site of Pb. In this research, there was no rat death during the experiment process. All animal were healthy and in normal behavioral performance during Pb and moringa extract administration.

This study results were similar to

previous study, which reported that Pb accumulation in the liver could be overcome with administering moringa leaves extract, as the extract is rich in polyphenols and flavonoids, which have metal chelation character. However, previous study used methanol extract.<sup>8</sup> Biochemically, the basic mechanism of lead toxicity is the capacity of lead to bond to crucial molecules in biologic systems, inhibiting their function through various pathways. From researches, the mechanism of lead toxicity occurs through its binding to sulfhydryl groups on proteins, mimicking and competing with calcium ions (Ca<sup>2+</sup>), and affecting the immune system by causing inflammation.<sup>14,15,16</sup> Lead interferes with several biochemical processes by binding to sulfhydryl groups and other nucleophilic functional groups and contributing to oxidative stress.<sup>17</sup> A researcher stated that the main mechanism for the toxic effect of lead is due to its combination with sulfhydryl groups in proteins, thereby inhibiting sulfhydryl dependent enzymes or having sulfhydryl groups.<sup>16</sup>

GSH production is regarded as the early array of protection contrary to oxidative damage and free radical formation. GSH serves as a scavenger and co-factor in metabolic detoxification. GSH contains a carboxylic group, an amino group, a sulfhydryl group and two peptide bonds in the act of the reaction location for lead. GSH functional group, -SH, shows a vital role in the binding of lead. Some studies have shown that GSH is depleted in lead-exposed rats' brain, liver, and eye lenses.<sup>15</sup> As such, it could be concluded that lead's inhibition effect on antioxidant enzymes and glutathione seems to damage the cells antioxidant defenses and make them highly vulnerable to oxidative attack.

Accumulating evidence has proven that lead exposure results in oxidative stress by promoting the formation of reactive oxygen species (ROS) and attenuating the cells' antioxidant defense systems. The decrease of the cell's main sulfhydryl stores appears to be vital indirect means for redox-inactive metal-induced oxidative stress. If lead depressed GSH, the GSH synthesis system begins to make further GSH from cysteine through the -glutamyl cycle.<sup>15</sup> GSH is generally not available

adequately, if GSH persistently decrease due to chronic Pb exposure. Some enzymes in the defense system of antioxidant can secure against this disproportion but the enzymes also become inactive because of the lead direct tie to the active site of the enzyme, if the site contains a sulfhydryl or thiol group e.g. ALAD. Furthermore, zinc that normally functions as a cofactor for numerous enzymes could be substituted by lead, rendering the enzyme inactive.<sup>15</sup>

In the enzymatic pathway, Pb decreases antioxidant defense by modifying the activity and expression of antioxidant enzymes.<sup>16</sup> Numerous harmful effects caused by lead in living systems have been associated with one or both of the covalent interactions of lead (Pb<sup>2+</sup>) with sulfhydryl groups (SH) of the antioxidant defense system, which usually protects against free radical toxicity, including scavenger enzymes like glutathione peroxidase (GPx), glutathione-S transferase, superoxide dismutase (SOD), and catalase (CAT), by replacing zinc ions which act as important cofactors at their catalytic sites and inactivate them.<sup>17</sup>

Lead has also been shown to increase and decrease the antioxidant enzymes catalase (CAT) levels and glutathione peroxidase (GPx). Furthermore, the effect of Pb<sup>2+</sup> can be seen in increasing the production of reactive oxygen species (ROS) by inducing oxidative stress by producing an imbalance between the production of free radicals in tissue and cellular components and the ability of antioxidant enzymes to detoxify highly reactive intermediates that cause damage to membranes, DNA and proteins.<sup>17</sup>

This study has proven that 50% ethanol extract of Moringa leaves ameliorated oxidative stress induced by acute exposure to Pb in the liver of Wistar rats. Previous studies on the antioxidant properties of Moringa leaves have obtained similar results, including in organs other than the liver. Previous research concluded that administration of Moringa leaf extract can reduce lipid peroxidase and increase SOD activity.<sup>18</sup> Another research documented that Moringa leaves extract ameliorated peroxidation damage by increasing SOD and GPx activities and decreased MDA and ROS levels.<sup>19</sup>

Moringa leaves contain great

polyphenolic compounds, including flavonoids and phenolic acids. Flavonoids are produced by plants to protect from the infection of microbes. They have a benzopyrone circle as a general form. Chronic diseases related to oxidative stress, such as cardiovascular disease and cancer, could be prevented by consuming flavonoids. Moringa leaves rich of flavonoids. The major flavonoids identified in Moringa leaves are myrecetin (5.8 mg/g), quercetin (0,207 mg/g), and kaempferol (7.57 mg/g).<sup>6</sup>

A sub-group of phenolic compounds, these are phenolic acids, originally from hydroxybenzoic acid, hydroxycinnamic acid, that are naturally found in plants, and they have antioxidant, anti-inflammatory, antimutagenic, and anticancer properties. In dry Moringa leaves, gallic acid was the amplest. Its concentration was 1.034 mg/g dry weight. The concentrations of chlorogenic acids ranged from 0.018 to 0.489 mg/g dry weight, whereas caffeic acid was 0.409 mg/g dry weight.<sup>6</sup>

Flavonoids can prevent injury due to free radicals and stabilize reactive oxygen species (ROS) which can bind to free radicals that cause degenerative diseases, by deactivating free radicals.<sup>20</sup> Flavonoids act as antioxidants because they have hydroxyl groups that can donate hydrogen atoms to free radical compounds and stabilize ROS and have hydroxyl ketone groups that have a role as metal chelators that have function as catalysts in lipid peroxidation.<sup>21</sup>

There are some limitations of this study. The first limitation is that this study didn't consider antioxidants contents of 50% ethanol extract Moringa oleifera leaves when decided treatment dose in the treatment groups. We only consider the extract dose from previous study. The second, this study didn't investigate other mechanism of Pb poisoning in the liver, such as inflammation pathway of Pb poisoning. The third, this study didn't measure Pb level in the liver. Future study is needed to make formulations considering antioxidant contents of the extract, other Pb poisoning mechanism besides stress oxidative, and the role of 50% ethanol extract of Moringa leaves as Pb chelator. Despite some weaknesses, this study proves that 50% ethanol extract of

Moringa leaves can reduce Pb toxicity in the liver by reducing oxidative stress. The implication of this study finding is that prevention of progressive liver damage due to Pb poisoning can be achieved by administration of Moringa leaves.

## CONCLUSION

This study concludes that 50% ethanol extract of moringa leaves doses 250, 500 and 1.000 mg/kgBW/day orally for 14 days ameliorates lead-induced oxidative stress in rat liver. This amelioration was seen in both enzymatic and non-enzymatic pathways. The minimum effective dose of 50% ethanol extract of Moringa leaves administration was 1.000 mg/kgBB/day orally for 14 days.

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## DISCLOSURE

The author reports no conflicts of interest in this work. The funding provider doesn't intervene in the research protocol development, research implementation, and publication process.

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## AUTHOR CONTRIBUTIONS

All authors contribute in all phase of this study, from literature searching, protocol development, research permit,

research implementation, data collection and analysis, study result reporting, and journal article writing processes. This article has been approved by all authors.

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**Letter of Acceptance**  
04 November 2022

**Dear: Agung Saprasetya Dwi Laksana<sup>1\*</sup>, Lily Kusumasita<sup>2</sup>, Falah Faniyah<sup>3</sup>**

<sup>1</sup>Epidemiology and Environmental Toxicology Division, Department of Public Health and Community Medicine, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Jawa Tengah, Indonesia

<sup>2</sup>Occupational Medicine Division, Department of Public Health and Community Medicine, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Jawa Tengah, Indonesia

<sup>3</sup>Department of Pediatrics, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Jawa Tengah, Indonesia

\*Corresponding author: [agung.laksana@unsoed.ac.id](mailto:agung.laksana@unsoed.ac.id)

I am very excited to accept your paper entitled:

**“Ameliorative effect of 50% ethanol extract of moringa leaves (*Moringa oleifera* Lam.) on lead-induced oxidative stress in the liver of male wistar rat model.”**

Your paper will be published in the issue of of Vol. 11 Number 3, 2022.

<http://dx.doi.org/10.15562/bmj.v11i3.3728>

(Online Link: <http://balimedicaljournal.org/index.php/bmj/article/view/3728>).

And it usually takes 2 to 4 months for your journal to show up at Google Scholar, but if you need it fast, you may add it up manually using your google scholar account. The CrossRef and DOI number usually activate in 3 until 6 months.

Bali Medical Journal is indexed in Web of Sciences, Scopus, and many other indexing organization: <http://balimedicaljournal.org/index.php/bmj/pages/view/indexing>

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| 13. Sherpa/Romeo                                      | 26. UDL-Edge (Malaysia)                               |

Please do not hesitate to contact us if you need anything. It has been a pleasure for us to proofread and edit your work, and we are looking forward to your colleagues and your other papers in the near future.

Agreed/Menyetujui by:

**Menyetujui,**  
  
**Prof. Dr. dr. Sri Maliawan, SpBS (K)**  
Editor in Chief

**Menyetujui,**  
  
**Prof. Dr. Ir. Ida Bagus Putra Manuaba, MPhil**  
Associate Editor



Agung Saprasetya Dwi Laksana 1 &lt;agung.laksana@unsoed.ac.id&gt;

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**Revision Required [BaliMedJ] [Manuscript ID:3728]**

12 messages

**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>

Thu, Oct 27, 2022 at 7:32 PM

To: agung.laksana@unsoed.ac.id, lily.burkon@unsoed.ac.id, falahfaniyah@unsoed.ac.id

Cc: I Gede Putu Supadmanaba &lt;supadmanaba@gmail.com&gt;, violinwedayani@gmail.com

Dear Authors,

Thank you for submitting your article entitled: "**Correlation of Erythroferrone and Hepcidin Hormones to Iron Status Levels in Patients with Iron Deficiency**"

Based on our author guidelines, Your article fulfilled the minimal required structure,

<https://www.balimedicaljournal.org/index.php/bmj/pages/view/authorguidlines>

In order to have a better-structured article, we suggest you edit based on a checklist and the collection in our archive (<https://www.balimedicaljournal.org/index.php/bmj/issue/archive>).

According to the new International regulation, please fulfill the requirements below:

1. Ethical clearance number/statement and/or informed consent at the end of the manuscript. Please described the patient's consent for publication in the disclosure section (**confirmed**).
2. Please state your conflict of interest in the paper (**confirmed**).
3. Please state the funding (if any) in your paper. (**confirmed**)
4. Please state each author's contribution (**confirmed**).
5. We detected 260 critical grammatical errors based on our proofreading application.
6. Please sort keywords alphabetically
7. Please add the inclusion and exclusion criteria at the method section

According to our reviewers, your article need **Moderate Revision**. Attached is the commentary file from our reviewers. Please read it carefully and revised your manuscript accordingly, and type the revision on the attached manuscript below.

Please revise your article and send it back to us in **7 days (November 3<sup>rd</sup>, 2022)**

In addition, I do need to remind you that Bali Medical Journal is free to submit and Open Access for our readers. However, if your manuscript is accepted for publication, as the author, you will be charged **1,100 USD for APC** included for **proofreading and editing (Formatting, Lay outing, and Galey)**.

For revising your article, we offer you editing and revising assistance which is provided by our official editing partner REVISE and according to your revision status, it will cost **150 USD**. Please confirm if you agree with this information.

Thank you for trusting us with your hard work and we are looking forward for your response.

Warm regards,

Executive Editor BaliMedJ

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**Bali Medical Journal (BaliMedJ)**

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Fri, Oct 28, 2022 at 11:12 AM

Dear Editor,  
Thank you very much for the information.  
Attached is our manuscript that has been revised according to your requirements.  
1. Keywords have been sorted alphabetically  
2. the inclusion and exclusion criteria have been added at the methods section (experimental procedure)  
3. Regarding 260 critical grammatical errors, we agree with your offer.

We are unable to pay 1100 for APC. Due to the COVID-19 pandemic, there is no support from the university for the publication funding  
Could we get a 50% rebate for this manuscript?

Thank you for your understanding. Looking forward to hearing from you soon.

Best regards,  
Authors  
[Quoted text hidden]

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**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>  
To: Agung Saprasetya Dwi Laksana 1 <agung.laksana@unsoed.ac.id>

Fri, Oct 28, 2022 at 1:05 PM

Dear Author

Regarding your request for a discount on the APC, we can clarify that our journal is Open Access and free for submission. According to our regulation, the accepted article must pay the Article Processing Charge and Proofread with a total of 1,100USD without considering the author's origin.

After considering your article and its importance in the medical field, we have a discount policy for your article, so you only have to pay **800 USD** for the Article Processing Charge and **100 USD** for Proofread (a total of **900 USD**) for your manuscript. Thank you for your consideration. **Please confirm if you agree with this information.**

Warm regards  
Executive Editor BaliMedJ  
[Quoted text hidden]

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Fri, Oct 28, 2022 at 9:36 PM

Dear Executive Editor BaliMedJ  
I agreed to pay a total of 900 USD. Thank you for your kindness.

In this email, I also would like to clarify that in your first email, you wrote:  
"Thank you for submitting your article entitled: **"Correlation of Erythroferrone and Hepcidin Hormones to Iron Status Levels in Patients with Iron Deficiency"**  
The manuscript title is not my manuscript. My manuscript title is **"Ameliorative effect of 50% ethanol extract of moringa leaves (Moringa oleifera Lam.) on lead-induced oxidative stress in the liver of male Wistar rat model"**.  
However, the manuscript you attached to your email was correct. The attachment was my manuscript.

Thank you very much for your kind attention. Looking forward to hearing from you soon

Yours sincerely,  
Authors

[Quoted text hidden]

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**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>  
To: Agung Saprasetya Dwi Laksana 1 <agung.laksana@unsoed.ac.id>

Sat, Oct 29, 2022 at 7:12 AM

Dear Author

After considering the suggestion from reviewers and also the quality of your manuscript (assisted by REVISE), we decided to **accept your manuscript for publication with moderate revisions**.

Attached below is the **invoice** for your article. Please use the rate from your bank when you want to pay in IDR. Also **please send us the proof of your payment through this email**, so we can process your article for publication. Congratulations on the acceptance of your article. We are looking for your future publication.

Best Regards

BMJ Editor  
[Quoted text hidden]

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Wed, Nov 2, 2022 at 11:28 AM

Dear Editor,  
Please kindly check the payment proof. We are waiting for the galley proof session.  
Thank you.

Best regards,  
Author  
[Quoted text hidden]

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287K

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**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>  
To: Agung Saprasetya Dwi Laksana 1 <agung.laksana@unsoed.ac.id>

Thu, Nov 3, 2022 at 7:49 AM

Dear Authors,

We have received your payment and we would like to inform you that your manuscript is now currently being processed by our reviewer and editor.

The process of articles by reviewers and editors takes 2-4 weeks after the LoA is sent to the author,

The LoA will be sent 5-7 days after the author completes the payment

Please patiently wait until we send you the revised version of your manuscript.

Thank you for trusting us with your hard work.

Best regards  
Editorial Team  
[Quoted text hidden]

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Sat, Nov 5, 2022 at 9:10 AM

Dear Editor,  
Thank you very much for your information.

Best regards,  
Authors  
[Quoted text hidden]

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**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>  
To: Agung Saprasetya Dwi Laksana 1 <agung.laksana@unsoed.ac.id>

Fri, Nov 11, 2022 at 8:48 AM

Dear Authors

With this email attached several documents of your submitted article entitled: "**Ameliorative effect of 50% ethanol extract of moringa leaves (*Moringa oleifera* Lam.) on lead-induced oxidative stress in the liver of male wistar rat model**"

- Final Edited Manuscript
- Final commentary
- Plagiarism Report of Original Manuscript
- Letter of Acceptance

The plagiarism reports of your original manuscript are 20%, which has already fulfilled the originality criteria. Our editor has fixed some sections in your manuscript according to the reviewer's suggestion.

**Please let us know if you are already satisfied** with the current final revised manuscript. If you approved this manuscript, your article will be processed for the galley version and published in Bali Medical Journal.

**If there is a revision from the author, please return the revised manuscript within 3 days (November 14th, 2022),** and type the revision on the attached manuscript below.

Inaccuracy in sending the revised manuscript will affect the time of publication.

We're looking forward to your progress, congratulations and good luck.

Best Regards  
Editorial Team

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#### 4 attachments

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Sun, Nov 13, 2022 at 4:53 PM

Dear Editor,  
I added "Jenderal Soedirman University" at the acknowledgement session. Beside this, I approved the manuscript. Thank you very much and we are waiting for the galley proof session.

Best regards,  
Authors  
[Quoted text hidden]

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**Editor Bali Medical Journal** <editorbalimedicaljournal@gmail.com>  
To: Agung Saprasetya Dwi Laksana 1 <agung.laksana@unsoed.ac.id>

Sun, Dec 4, 2022 at 8:36 PM

Dear author,  
Thank you for the revised manuscript  
Your article will be processed for the galley version and published in Bali Medical Journal.

Best regards  
BMJ Editor  
[Quoted text hidden]

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**Agung Saprasetya Dwi Laksana 1** <agung.laksana@unsoed.ac.id>  
To: Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>

Tue, Dec 6, 2022 at 5:29 AM

Dear BMJ Editor,  
Thank you for the information.

Best regards,  
Authors  
[Quoted text hidden]