Anti-obesity activity of *Erythrina subumbrans* (Hassk.) Merr leaves extract in high fructose-induced obesity in Wistar rats

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**Abstract**

**Introduction:** Obesity is known to lead to the development of metabolic disorders and *Erythrina subumbrans* (Hassk.) Merr has shown the potential in alleviating some diseases. This study aimed to assess the anti-obesity activity of the ethanol extract of *E. subumbrans* leaves (EES) in obese Wistar albino rats.

**Methods:** The rats were randomly assigned to eight groups (n = 5): normal group (no obesity inducement, treated with standard feed); high fructose (60%)-induced obesity (obesity inducement, no treatment); control-1 group (obesity-induced, treated with orlistat 10.8 mg/kg BW); control-2 group (obesity-induced, treated with Isprinol 45 mg/kg); control-3 group (obesity-induced, treated with metformin 45 mg/kg); three test groups (obesity-induced, treated with EES 100, 200, and 400 mg/kg, respectively). The obesity inducement was conducted for 7 weeks. The rats were observed for body weight (BW), feed residue, feces index, triglycerides, organ index, and leucocyte profile.

**Results:** The screening for secondary metabolites in EES revealed the presence of flavonoids, alkaloids, saponins, polyphenols, and tannins. EES decreased the percentage of BW gain, appetite, organ index (spleen), total fat, triglycerides, and leucocyte profile of the rats.

**Conclusion:** The ethanol extract of the leaves of *E. subumbrans* has the potential to be developed as an anti-obesity agent, although the molecular mechanism of its pathway modulation is still unknown.

**Implication for health policy/practice/research/medical education:** This study may support the development of *Erythrina subumbrans* (Hassk.) Merr leaves as an active component of an anti-obesity drug.

predicted to associate with IR by altering the insulin signaling pathway in adipose tissue and skeletal muscle (10).

Alleviation of diseases using plants is challenging, therefore discovering plant-based drugs is considered important and worth studying. Plants that contain secondary metabolites are potential due to their various pharmacology activities, for example, the barks, twigs, and roots of *Erythrina subumbrans* have been announced to contain alkaloids, pterocarpan, flavonanes, and triterpenes (11-13).

Alkaloids have advantages on human health, e.g., anticancer, anti-inflammatory, antihypertensive, anti-diabetic, and anti-oxidant activities. These compounds influence the human central nervous system and target nucleic acid, DNA, and RNA (14). A group of polyphenolic compounds, e.g., flavonoids, such as naringenin, could affect various immune signaling pathways, enhance endogenous enzyme activities, and block NF-kB activity, hence suppressing the expression of proinflammatory cytokines (15,16). Flavonoids were announced for their impact on obesity via several mechanisms such as decreasing food intake and fat absorption, elevating energy expenditure, altering lipid metabolism, or affecting intestinal microbiota (17,18). Moreover, studies on animal models revealed that polyphenols reduce obesity, body weight, and blood triglycerides, by increasing fat use, energy expenditure, and glucose balance (19).

Previous studies reported various pharmacological activities of *Erythrina* plants. For example, the leaves and barks of *E. variegata*, had shown analgesic and anti-inflammatory activities (20-22); the leaves extract of *E. variegata* exhibited hypolipidemic activity (23); the stem bark extract of *E. abyssinica* could decrease body weights, triglyceride levels, and sterol levels in *Drosophila melanogaster* flies exposed to coconut diet (24).

Studies on *E. subumbrans* are still limited; however, the twigs and roots have shown anti-diabetic and antimicrobial activities (13), whereas the leaves were reported to exhibit an anti-inflammatory activity on carragenan-induced edema in rats (25,26). Thus, considering the secondary metabolites of *E. subumbrans* may also have an impact on obesity, our study aimed to assess the anti-obesity activity of *E. subumbrans* leaves extract in high fructose-induced obesity in Wistar albino rats.

**Materials and Methods**

**Plant**

The leaves of the plant were collected from Ciamis, West Java, Indonesia, and verified by a botanist at the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Indonesia. The plant samples were confirmed as *Erythrina subumbrans* (Hass.) Merr. (Fabaceae) (Herbarium sample number 35/HB/2023) and matched those illustrated in Naturalis Biodiversity Center (NL)-Botany (https://www.gbif.org/occurrence/2517594190).

**Extract preparation**

The leaves were cleaned, washed, and dried indoors for several days. The dried leaves were ground, sieved (mesh 4/18), soaked in ethanol for 3×24 hours at 25-26 °C, filtered, and the solvent was evaporated at 50-60 °C to a thick consistency. The resulting ethanol extract of *E. subumbrans* is abbreviated as EES. EES was examined to ensure its quality as required by the Indonesian Herbal Pharmacopoeia (27) and screened for its secondary metabolite content by following a previous method (25).

**Animals**

Forty male Wistar rats, obtained from an official animal breeding farm in Bandung, West Java, Indonesia, were randomly grouped into eight cages at 25-26 °C under a 12 hours light, 12 hours dark cycle, 55 % relative humidity (RH), with standard feed (containing carbohydrate, vegetable protein, and vegetable fat) and water ad libitum for 1-week adaptation. The rats' health and behavior were observed every day during the adaptation and *in vivo* study. The cages were cleaned every three days and the feces were collected and weighed every ten days. The animal handling was approved by the Research Ethics Committee, Padjadjaran University, Indonesia (https://kep.unpad.ac.id/with the approval documents number 506/UN6.KEP/EC/2022 and 903/UN6.KEP/EC/2023). No animal was found dead during the study.

**Assessing anti-obesity activity**

The *in vitro* and animal studies were performed at the Pharmacology Laboratory of Universitas Bhakti Kencana, Bandung, West Java, Indonesia from July 2022 to June 2023. The rats were randomly grouped into (1) the normal group (treated with standard feed); (2) high fructose-induced obesity (treated with fructose 60%); (3) control-1 group (treated with orlistat 10.8 mg/kg BW); (4) control-2 group (treated with Isprinol 45 mg/kg BW); (5) control-3 group (treated with metformin 45 mg/kg BW); (6) test-1 group (treated with EES 100 mg/kg BW); (7) test-2 group (treated with EES 200 mg/kg BW); (8) test-3 group (treated with EES 400 mg/kg BW). The control and EES groups were obesity-induced groups using fructose 60% for 7 weeks.

The rats were observed for BW, feed residue (appetite), and fecal weight at D0, D10, D20, D30, and D60. Moreover, the blood glucose and serum TNF-alpha levels were also measured. Blood glucose was measured at D0, D30, and D60 using the EasyTouch blood glucose meter (28). Results were expressed as the % change in blood glucose
level compared to the initial level (D0). The triglyceride levels were measured at D0 and D60 using Proline Triglycerides FS 10 (https://proline.co.id/our-product/reagents/clinical-chemistry/triglycerides-fs-10/). Results were expressed as the % change in triglyceride level compared to the initial level (D0).

At D60, the rats were sacrificed using carbon dioxide and analyzed for their organ index (kidney, heart, lung, liver, spleen, and testis), total fat, and immune profile (neutrophil, lymphocyte, monocyte) by following the Regulation of the Indonesia National Agency of Drug and Food Control (https://www.pom.go.id/new/home/en). Total fat was determined by separating the fat layer on the retroperitoneal, epidydimal, and perirenal (depicted in Figure 1).

Statistical analysis
SPSS for Windows was utilized to analyze the data. Significant differences between groups were analyzed using one-way ANOVA continued with the post-hoc Tukey test. All data are presented as the mean ± SD; P < 0.05 indicates a significant result.

Results

Phytochemicals and standard quality of EES
The examination of the extract confirmed its high quality (presented in Table 1) as required by the Indonesian Herbal Pharmacopoeia. The screening of secondary metabolites in EES revealed the presence of flavonoids, alkaloids, saponins, polyphenols, and tannins.

Effects of EES on the % weight gain of rats
At the end of the study (D60), all rats demonstrated an increase in body weight; however, treating the rats with orlistat, metformin, and EES resulted in a lower % weight gain compared to the rats in the obesity-induced group. Thus, EES positively possesses anti-obesity activity as proven by the significant difference of % weight gain between rats treated with EES compared to the rats in the obesity-induced group (without treatment) (marked with # in Table 2). The anti-obesity activity of all doses of EES was similar to that of orlistat; however, only an EES dose of 400 mg/kg BW showed a comparable value of % weight gain with that of metformin.

Table 1. The quality of the ethanol extract of *Erythrina subumbrans* (EES)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amount in percentage (%)</th>
<th>Standard quality required by the Indonesian Herbal Pharmacopoeia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>3.84</td>
<td>≤10</td>
</tr>
<tr>
<td>Total ash</td>
<td>7.25</td>
<td>≤7.5</td>
</tr>
<tr>
<td>Undissolved ash</td>
<td>0.75</td>
<td>≤1.5</td>
</tr>
<tr>
<td>Water-dissolved essence</td>
<td>25</td>
<td>≥18</td>
</tr>
<tr>
<td>Ethanol-dissolved essence</td>
<td>13</td>
<td>≥10</td>
</tr>
</tbody>
</table>

Table 2. Effect of the ethanol extract of *Erythrina subumbrans* (EES) on the body weight of Wistar rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Mean BW (in g ± SD)</th>
<th>% Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
<td>D10</td>
</tr>
<tr>
<td>Normal group</td>
<td>182.8 ± 5.9</td>
<td>188.1 ± 19.2</td>
</tr>
<tr>
<td>Obesity-induced group</td>
<td>183.3 ± 5.9</td>
<td>196.8 ± 15.9</td>
</tr>
<tr>
<td>Orlistat group</td>
<td>191.0 ± 4.3</td>
<td>196.2 ± 8.1</td>
</tr>
<tr>
<td>Metformin group</td>
<td>184.3 ± 12.3</td>
<td>184.3 ± 11.6</td>
</tr>
<tr>
<td>EES 100 mg/kg BW</td>
<td>180.0 ± 3.6</td>
<td>186.2 ± 8.3</td>
</tr>
<tr>
<td>EES 200 mg/kg BW</td>
<td>180.8 ± 5.6</td>
<td>194.2 ± 7.7</td>
</tr>
<tr>
<td>EES 400 mg/kg BW</td>
<td>180.5 ± 5.9</td>
<td>180.7 ± 7.5</td>
</tr>
</tbody>
</table>

* Indicates a significant difference compared to the normal group (P < 0.05); # indicates a significant difference compared to the fructose 60%-induced obesity (P < 0.05); α indicates a significant difference compared to the orlistat group (P < 0.05); β indicates a significant difference compared to the metformin group (P < 0.05)
EES Increases the Appetite

All doses of EES affected the appetite of the rats as indicated by the decrease of feed residue and the increase of fecal weight (Table 3).

On D60 (the last day of the experiment), rats in all groups gained their appetite as shown by the decrease in the feed residue (uneaten feed) as follows: normal group 30%, obesity-induced group 71.4%, orlistat group 73.1%, EES 100 mg/kg 76.8%, and EES 400 mg/kg 63.6%. The rats treated with EES 200 mg/kg BW showed the smallest increase in appetite, which was proven by the smallest percentage decrease of the feed residue of 9.5%.

We also calculated the fecal weight of the rats and found that different effects resulted as follows: an increase (21.7%) of fecal weight in the normal group, a decrease (10%) in the obesity-induced group, an increase (26.3%) in the orlistat group, and an increase of fecal weight in EES 100 mg/kg (130%), 200 mg/kg (47.4%), and 400 mg/kg (54.5%), respectively.

On D60, the decrease of feed residue and the increase of fecal weight of rats treated with EES dose 200 mg/kg was significantly different from those of the obesity-induced rats (P<0.05). Interestingly, the rats treated with orlistat also showed similar results.

EES exhibited no alteration of the total fat

All doses of EES did not alter the total fat of rats as presented in Table 4. The statistical analysis resulted in insignificant differences (P>0.05) between EES treatment rats compared to the rats in other groups.

EES did not affect the organ index

All doses of EES demonstrated no effect on the kidneys and lungs of the rats; however, the heart and the liver of the rats treated with an EES dose of 400 mg/kg showed a significant increase compared to the rats treated with orlistat, not with the fructose-induced obese rats. Nevertheless, the spleen of the rats treated with an EES dose of 200 mg/kg showed a significant increase compared to the fructose-induced obese rats and rats in the normal group (Table 5).

EES reduced the blood glucose

The rats in all groups, with the exception of the normal group, underwent a decrease in their blood glucose levels. Statistical analysis revealed no significant difference compared to the obesity-induced rats (Table 6).

EES reduced triglyceride levels

Rats in all groups, with the exception of the normal group, underwent a decrease in their triglyceride levels. Statistical analysis revealed no significant difference compared to the obesity-induced rats (Table 7).

Table 3. Effect of the ethanol extract of Erythrina subumbrans (EES) on the appetite of Wistar rats (in terms of feed residue and fecal weight)

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>Observation days</th>
<th>Feed residue</th>
<th>Fecal weight</th>
<th>Feed residue</th>
<th>Fecal weight</th>
<th>Feed residue</th>
<th>Fecal weight</th>
<th>Feed residue</th>
<th>Fecal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>D10</td>
<td>0.5 ± 0.1*a</td>
<td>2.3 ± 0.1</td>
<td>0.3 ± 0.1*a</td>
<td>2.6 ± 0.0</td>
<td>0.6 ± 0.1*a</td>
<td>2.6 ± 0.5*a</td>
<td>0.1 ± 0.0*a</td>
<td>2.8 ± 0.4*a</td>
</tr>
<tr>
<td>Obesity-induced group</td>
<td>D20</td>
<td>4.2 ± 0.1*a</td>
<td>2.0 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.3 ± 0.0</td>
<td>0.8 ± 0.2*a</td>
<td>2.6 ± 0.4</td>
<td>1.2 ± 0.0*a</td>
<td>1.8 ± 0.3*a</td>
</tr>
<tr>
<td>Orlistat group</td>
<td>D30</td>
<td>9.3 ± 0.2*a</td>
<td>1.9 ± 0.4</td>
<td>4.8 ± 0.2*a</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.0*a</td>
<td>3.2 ± 0.4*a</td>
<td>2.5 ± 0.3*a</td>
<td>2.4 ± 0.4*a</td>
</tr>
<tr>
<td>EES 100 mg/kg BW</td>
<td>D60</td>
<td>9.5 ± 0.0*a</td>
<td>1.0 ± 0.3*a</td>
<td>2.5 ± 0.0*a</td>
<td>1.9 ± 0.2*a</td>
<td>2.0 ± 0.3*a</td>
<td>2.8 ± 0.5</td>
<td>2.2 ± 0.0*a</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>EES 200 mg/kg BW</td>
<td></td>
<td>4.2 ± 0.0*a</td>
<td>1.9 ± 0.4</td>
<td>2.2 ± 0.2*a</td>
<td>1.9 ± 0.4*a</td>
<td>2.3 ± 0.2*a</td>
<td>2.5 ± 0.3*a</td>
<td>3.8 ± 0.0*a</td>
<td>2.8 ± 0.4*a</td>
</tr>
<tr>
<td>EES 400 mg/kg BW</td>
<td></td>
<td>9.9 ± 0.1*a</td>
<td>1.1 ± 0.3*a</td>
<td>3.6 ± 0.2*a</td>
<td>2.5 ± 0.2</td>
<td>2.5 ± 0.0*a</td>
<td>2.9 ± 0.4</td>
<td>3.6 ± 0.2*a</td>
<td>1.7 ± 0.3*a</td>
</tr>
</tbody>
</table>

*a Indicates a significant difference compared to the normal group (P<0.05); # indicates a significant difference compared to the fructose 60%-induced obesity (P<0.05); α indicates a significant difference compared to the orlistat group (P<0.05). Orlistat was used as the control drug.

Table 4. The effect of the ethanol extract of Erythrina subumbrans (EES) on the total fat of albino Wistar rats

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>The mean of % fat weight ± SD</th>
<th>Total fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retroperitoneal</td>
<td>Perirenal</td>
</tr>
<tr>
<td>Normal group</td>
<td>1.5 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Obesity-induced group</td>
<td>1.7 ± 0.6</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Orlistat group</td>
<td>1.4 ± 0.8</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>Metformin group</td>
<td>1.1 ± 0.2</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>EES 100 mg/kg</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>EES 200 mg/kg</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>EES 400 mg/kg</td>
<td>1.7 ± 1.0</td>
<td>1.3 ± 0.7</td>
</tr>
</tbody>
</table>

http://www.herbmedpharmacol.com
EES did not alter the leucocyte profile

EES in all doses did not significantly influence the number of neutrophils and monocytes compared to the obesity-induced rats. However, the lymphocytes of rats treated with EES were significantly lower than those of the obesity-induced rats but higher than the Isprinol and metformin groups. The data was used to calculate the neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR) at D60 using the following formulas (29):

\[
\text{Neutrophil-to-lymphocyte ratio (NLR)} = \frac{\text{neutrophil count}}{\text{lymphocyte count}}
\]

\[
\text{Monocyte-to-lymphocyte ratio (MLR)} = \frac{\text{monocyte count}}{\text{lymphocyte count}}
\]

The results are presented in Table 8.

### Discussion

This study assesses the anti-obesity activity of the ethanol extract of *E. subumbrans* (Hassk.) Merr leaves (EES) in obese Wistar rats. Orlistat (synonym: tetrahydrolipstatin) has been approved by the FDA as an anti-obesity agent. This drug works by inactivating gastric and pancreatic enzymes that are responsible for fat absorption.
lipases, enzymes that hydrolyze fat taken from food to produce free fatty acids and monoglycerides (30). Orlistat has shown mild adverse effects thus it becomes the first line of therapy in patients with obesity (31). It was reported for its effect in reducing BP, improving insulin sensitivity and lipid profiles, and decreasing intestinal fat absorption (32). Isprinol (synonym: methisoprinol or inosine pranobex) is not an anti-obesity drug. It works as an immunomodulator by stimulating a Th1 cell-type response thus increasing the levels of proinflammatory cytokines. It is considered safe without serious adverse effects, but a few reported nausea in long-term use (33). Metformin works as an antidiabetic agent by reducing the production of glucose in the liver. This drug affects both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent (34).

Our results revealed that there was a significant decrease in the % weight gain between the rats treated with all doses of EES compared to the rats in the obesity-induced group (without treatment), indicating that EES may possess anti-obesity activity. This activity was similar to that of orlistat; however, only an EES dose of 100 mg/kg showed a similarity of activity with metformin.

Moreover, although the rats in all groups showed a decrease in appetite as shown by more than a 30% decrease in the feed residue, the rats treated with EES 200 mg/kg showed the smallest increase in appetite (9.5% decrease in the feed residue). At the end of the study, there was a significant decrease in feed residue and an increase in fecal weight of rats treated with an EES dose of 200 mg/kg compared with those of the obesity-induced rats (P<0.05) and similar to that of rats treated with orlistat.

Our study was in agreement with a previous anti-obesity study on *E. abyssinica* stem bark (24). Moreover, another *in vitro* study on an isolate of the stem bark of *E. abyssinica* confirmed its inhibitory activity towards pancreatic lipase, an essential enzyme in obesity (35).

All doses of EES demonstrated no effect on the kidneys, heart, and lungs of the rats indicating the safety of EES towards these organs; however, the spleen of the rats treated with an EES dose of 200 mg/kg showed a significant increase compared to both the obesity-induced and normal group rats.

The safety of EES was similar to a previous study of acute and subchronic toxicity of *E. senegalensis* obtained from Cameroon. A single dose of 300, 600, and 1,200 mg/kg/day given to rats for 28 days confirmed no hematological alteration, whereas the serum alkaline phosphatase, alanine transaminase, aspartate aminotransferase, total protein, total cholesterol, and triglycerides reduced significantly. Histological examination did not reveal changes in the liver and kidney (36).

Interestingly, the rats in the fructose 60%-induced obesity group demonstrated a significantly higher level of TNF-alpha at D60 compared to those of the normal group. Furthermore, EES did not significantly influence the number of neutrophils and monocytes compared to the obesity-induced rats; however, a significant difference in the lymphocytes was observed.

A recent study reported that the water extract of *E.
lysystemon bark strongly stimulated the production of anti-inflammatory cytokines such as IL-10 and IL-2 in RAW 264.7 cells, which confirmed the immunomodulatory activity of this plant (37).

Obesity is related to the incidence of low-grade chronic inflammation, revealing an association between metabolism and immunity. Pro-inflammatory cytokines are thought to work by reducing triglyceride synthesis via the alteration of peroxisome proliferator-associated receptor gamma (38). A previous study confirmed that a high-fat high-sucrose diet led to an increase in body fat accumulation and insulin resistance (39).

The NLR and MLR can be calculated to measure the level of inflammation. The higher values of NLR and MLR imply neutrophilia or lymphopenia, respectively. Some studies in humans have reported that NLR and MLR increase in patients with depressive episodes (29) and are linked to the severity of the illness (40).

Our results indicate that treatment with EES could gradually reduce the blood glucose levels of the rats although no significant differences could be seen in blood glucose levels between the rats treated with EES compared to the obesity-induced group. Moreover, EES reduced triglyceride levels at D60 compared to D0. In agreement with our results, the hypolipidemic activity of other Erythrina plants was also reported (23,24).

Many plants have been studied in animal models for their potential as anti-obesity (41-47). It was described that plant secondary metabolites may demonstrate their anti-obesity activity via several mechanisms, e.g., by decreasing the appetite, blocking pancreatic lipase, increasing thermogenesis and lipid metabolism, suppressing triglycerides in the liver and adipose tissue, suppressing appetite signals in the brain, elevating satiety, and epigenetic mechanisms (17-19,48). Furthermore, it was reported that quercetin, a well-known flavonoid contained in most plants, did not significantly decrease their anti-obesity activity via several mechanisms, e.g., by decreasing the appetite, blocking pancreatic lipase, increasing thermogenesis and lipid metabolism, suppressing triglycerides in the liver and adipose tissue, suppressing appetite signals in the brain, elevating satiety, and epigenetic mechanisms (17-19,48). Furthermore, it was reported that quercetin, a well-known flavonoid contained in most plants, did not significantly decrease their anti-obesity activity via several mechanisms, e.g., by decreasing the appetite, blocking pancreatic lipase, increasing thermogenesis and lipid metabolism, suppressing triglycerides in the liver and adipose tissue, suppressing appetite signals in the brain, elevating satiety, and epigenetic mechanisms (17-19,48).

Conclusion

An anti-obesity assessment of the ethanol extract of E. subumbrans (Hassk.) Merr leaves has been conducted in an animal model. The rats were induced obesity using fructose 60% per oral and provided evidence that the ethanol extract of EES has the potential to be developed as an anti-obesity agent as proven by a significant difference in % BW gain and triglycerides compared to the rats in the obesity-induced group. However, this study still lacks the molecular mechanism of which pathway is modulated by EES.

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Authors’ contributions

Data curation: Agus Sulaeman and Soni Muhsinin.

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Project administration: Agus Sulaeman.

Supervision: Sri Adi Sumiwi, Jutti Levita, and Yasmiwar Susilawati.

Validation: Sri Adi Sumiwi and Jutti Levita.

Writing—original draft: Elis Susilawati.

Writing—review & editing: Jutti Levita.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the first author upon reasonable request.

Conflict of interests

None to declare.

Ethics considerations

The animal handling was approved by the Research Ethics Committee, Padjadjaran University, Indonesia (https://kep.unpad.ac.id/ with the approval documents number 506/UN6.KEP/EC/2022 and 903/UN6.KEP/EC/2023).

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