

# Potential Antioxidant Root of Pasak Bumi (*Eurycoma longifolia* Jack) as Oxidative Stress Therapy in Obesity

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## Abstract

**Background:** Obesity is a chronic disease due to excess accumulation of body fat and is a major contributing factor to metabolic syndrome. In obesity, chronic inflammatory conditions occur especially in white fat which triggers oxidative stress. Many studied in obesity had proved patient with obesity have high level oxidative stress. Pasak bumi (*Eurycoma longifolia* Jack) is a plant that grows endemic in Indonesia, especially Kalimantan. Many studies have proven that there is a strong relationship between testosterone levels and the incidence of obesity and metabolic syndrome. Obese people have low plasma testosterone levels.<sup>7</sup> Pasak bumi contains saponins and tannins that can reduce the absorption of dietary fat and inhibit lipogenesis activity. Pasak bumi also contains flavonoids, several studies have reported that the flavonoid content in some plants can cause adipocyte differentiation inhibitory effects and as antioxidants to solving the problem of oxidative stress in obesity.

**Materials and Methods:** This study followed ethical consideration for animal research and had ethical approval from the ethics committee of the Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia with No.586/KEPK-FK ULM/EC/VI/2021. This study was an experimental study with posttest-only with control group design.

**Results:** EPB doses of 7.5-22.5 mg can significantly increase SOD levels in treated mice compared to the control group, with a maximum dose of 22.5 mg/kg BW of EPB.

**Conclusion:** EPB can reduce MDA and peroxide level in obese rats.

**Key Word:** Intrathecal; Bupivacaine; Buprenorphine; Nalbuphine; Postoperative analgesia.

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## I. Introduction

Obesity is a chronic disease due to excess accumulation of body fat and is a major contributing factor to metabolic syndrome. In obesity, chronic inflammatory conditions occur especially in white fat which triggers oxidative stress. Many studied in obesity had proved patient with obesity have high level oxidative stress which is characterized by an increasevarious parameters of free radicals and reactive oxygen species (ROS) products.<sup>1,2</sup> Oxidative stress triggered by obesity causes many problems of morbidity and mortality due to complications that occur.<sup>3-6</sup> For this reason, it is necessary to find an alternative treatment for obesity that is effective to solving the problems that arise due to obesity.

Pasak bumi (*Eurycoma longifolia* Jack) is a plant that grows endemic in Indonesia, especially Kalimantan. Many studies have proven that there is a strong relationship between testosterone levels and the incidence of obesity and metabolic syndrome. Obese people have low plasma testosterone levels.<sup>7</sup> Pasak bumi contains saponins and tannins that can reduce the absorption of dietary fat and inhibit lipogenesis activity.<sup>8,9</sup> Pasak bumi also contains flavonoids, several studies have reported that the flavonoid content in some plants can cause adipocyte differentiation inhibitory effects and as antioxidants to solving the problem of oxidative stress in obesity.<sup>10,11</sup> This study generally aims to analyze the potential of pasak bumi as an anti-oxidant in obesity. Specifically to analyze the effect of pasak bumi on the oxidative stress profile: levels of H<sub>2</sub>O<sub>2</sub>, SOD, Catalase, and MDA in obese rats.

## II. Material And Methods

This study followed ethical consideration for animal research and had ethical approval from the ethics committee of the Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia with

No.586/KEPK-FK ULM/EC/VI/2021. This study was an experimental study with posttest-only with control group design.

### **Material**

Pasak bumi (*E. longifolia Jack*) root, high fat and calorie feed, standard diet, aquadest, etanol 70%, PBS pH 7.4, Na-thiobarbiturate, HCL, TCA, EDAT, H<sub>2</sub>O<sub>2</sub>, dichromate, adrenaline, Na<sub>2</sub> CO<sub>3</sub>, acetic acid, ketazyl, gloves, mask. Rotary evaporator, analytical balance, measuring cup, blender, oral probe, glass apparatus, disposable syringe, vacutainer tube, cuvette, water bath, spectrophotometer, vortex, incubator, centrifuge.

### **Procedures**

**Preparation of experimental obese rats:** 4 month-old *Rattus norvegicus* giving high fat (45% fat) food for 8 weeks. Obesity was declared if the weight has increased 20% from normal rat weight of the same age

**Preparation of the ethanol extract 70% Pasak bumi root (EPB):** *Rattus norvegicus* obese were divided into 5 groups plus 1 group of normal rats as a normal control. The group of rats consists of: Normal rats as a control group (K); Obese rats + placebo/aquades (P1); obese rats + EPB dose of 7.5 mg/kg (P2); obese rats + EPB dose 15 mg/kg (P3); obese rats have been + EPB dose 22.5 mg/kg (P4); and obese rats +EPB dose of 30 mg/kg rat weight have been given for 28 days

### **Laboratory measurement**

**Measurement SOD, peroxidase (H<sub>2</sub>O<sub>2</sub>), catalase, and MDA assay from blood rats:** *R. norvegicus* were surgically and performed phlebotomy. 5 mL rats blood was centrifuged 8000 rpm for 20 minutes then serum was taken to measured antioxidant profile.

**Measurement SOD level:** Incubation was performed on 3 mL of a solution containing 0.05 M Na<sub>2</sub>CO<sub>3</sub>, 0.1 M EDTA pH 10.2. Furthermore, the solution was added 100 µL serum and 100 mL adrenaline with (3.10<sup>-4</sup>) BM 189 M. Initial absorption measurements (A0) was performed with a spectrophotometer at 480 nm wavelength. After that, the sample was incubated for 5 min at 30<sup>0</sup>C and got the absorbance (A1).

**Measurement peroksidase (H<sub>2</sub>O<sub>2</sub>) and catalase level:** Measurement of H<sub>2</sub>O<sub>2</sub> was using a spectrophotometer. At first, making a standard curve. A total of 20 mmol H<sub>2</sub>O<sub>2</sub> was added with 2 mL of dichromate:glacial acetic acid (1:3) mixture. Then the mixture was heated in boiling water for 10 min. Then the cooled mixture was measured for absorbance at a wavelength of 570 nm. The same procedure was done for 40, 60, 80, 100, 120, 140, 160 and 180 mmol H<sub>2</sub>O<sub>2</sub>. A graph was made between the absorbance on the Y-axis with levels of H<sub>2</sub>O<sub>2</sub> on the Xaxis to obtain a linear equation. Preparation of test solution was made with a total of 1 mL of serum was added 5 mL of PBS pH 7.4. A mixture of 1 mL was taken and added to 2 mL of dichromate:acetate (1:3) mixture and then wrapped in aluminum foil for 30 min. The mixed solution was heated using a water bath for 10 min at 100<sup>0</sup> C. The solution was cooled to room temperature. The solution was then transferred into the cuvette and measured its absorbance using UV-VIS at a wavelength of 570 nm

**Measurement MDA level:** The first thing to do was making MDA standard curve. As many as 0.05 mM MDA standard added 1 mL of distilled water, then placed in Eppendorf tube. Thereafter, 100 mL of 100% TCA, 100 mL sodium thiobarbituric 1%, and 250 mL HCl 1 N were added respectively. Then heated at 100<sup>0</sup> C for 20 min, and centrifuged 3500 rpm for 10 min. Subsequently, 450 mL supernatant was taken and the distilled water added to 3500 mL. Then read with the spectrophotometer with a maximum wavelength of 540 nm. The same thing was done to 0.025, 0.0125, 0.00625, 0.003125 and 1.56 x 10<sup>-5</sup> µM MDA. Then making graphs for the relationship between absorbance on the Y-axis and MDA levels on the X-axis to obtain a linear equation

### **Statistical Analysis**

Data were tested for normality and homogeneity. If normal, proceed with the Anova test analysis with a 95% confidence level and a tuckey HSD test. If it is not normal then a Kruskal Wallis nonparametric test is followed by Mann Whitney with a 95% confidence level.

## **III. Result**

### **SOD enzyme activity Level**

After administration of high fat and calorie feed for 28 days, rats wheight was increased average 10-34 mg. SOD enzyme activity in groups is presented in Fig 1. It can be seen that in group K0 (normal rats) SOD enzyme activity was lower than other groups except P4. Meanwhile, the P3 group who were given EPB at a dose of 22.5 mg/kgBW showed the highest SOD activity.

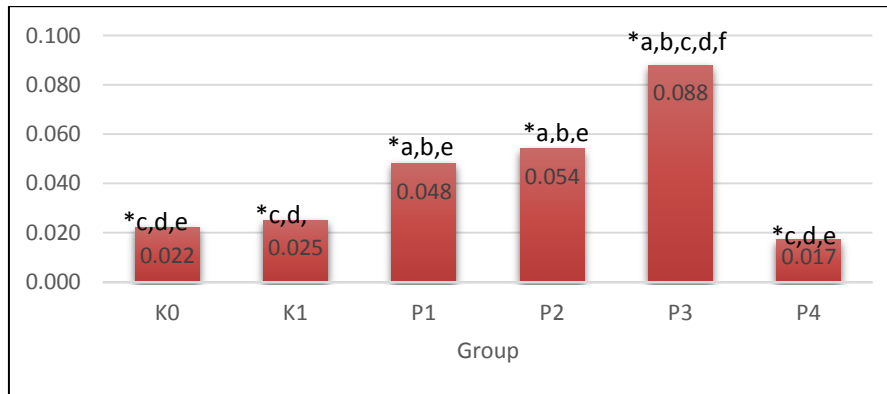


Fig 1. Superoxide dismutase (SOD) enzyme activity of *Rattus novergicus* after administration of *Eurycoma longifolia* Jack, extract ethanol 70%. Note K0=normal +placebo; K1 = high fat and calori feed +placebo; P1= high fat and calori feed + EPB 7.5 mg/kgBW; P2= high fat and calori feed +EPB 15 mg/kgBW; P3= high fat and calori feed +22.5 mg/kgBW; P4 = high fat and calori feed +30 mg/kgBW., a : there are significant differences compared with K0 group (p < 0.05); b : there are significant differences compared with K1 group (p < 0.05); c : there are significant differences compared with P1 group (p < 0.05); d : there are significant differences compared with P2 group (p < 0.05); e : there are significant differences compared with P3 group (p < 0.05); f : there are significant differences compared with P4 group (p < 0.05);

The results SOD enzyme activity of K0 (normal control) was significantly different from P1, P2, P3 but not with P4. Mann Whitney post hoc test results showed that the P3 group was significantly different from K1, P1, P2, and P4. This indicates that the dose of 22.5 mg/kgBW of EPB was able to increase the SOD enzyme activity of rats fed the high fat and calori better than other dose. Meanwhile, the highest dose of 30 mg/kgBW (P4 group) showed low SOD enzyme activity and this was not significantly different from K0 and P1 groups

#### Peroxide level

Fig.2 shows that peroxidase level in the groups. the P1 group which was given EPB at a dose of 7.5 mg/kgBW showed the highest levels of peroxide. The results of the normality statistical test obtained normal data distribution, so that it can be continued with the ANOVA test with a significance level of 95% to see the effect of giving EPB to the treatment group. The results of the ANOVA test showed p value = 0.000 which means that there were significant differences in all treatment groups. This indicates EPB administration have effect of on the peroxide levels of rats.

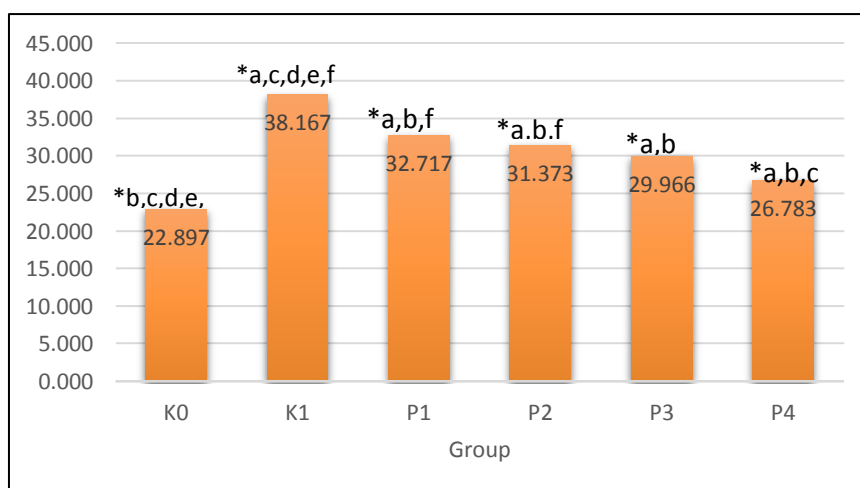


Fig 2. Peroxidase level of *Rattus novergicus* after administration of *Eurycoma longifolia* Jack, extract ethanol 70%. Note K0=normal +placebo; K1 = high fat and calori feed +placebo; P1= high fat and calori feed + EPB 7.5 mg/kgBW; P2= high fat and calori feed +EPB 15 mg/kgBW; P3= high fat and calori feed +22.5 mg/kgBW; P4 = high fat and calori feed +30 mg/kgBW., a : there are significant differences compared with K0 group (p < 0.05); b : there are significant differences compared with K1 group (p < 0.05); c:there are significant differences compared with P1 group (p < 0.05); d : there are significant differences compared with P2 group (p < 0.05); e : there are significant differences compared with P3 group (p < 0.05); f : there are significant differences compared with P4 group (p < 0.05);

The normal control group (K0) had peroxide levels that were significantly different from the all treatments group. K1 group who given high fat and kalori diet without EPB showed the highest peroxide level significantly compared to the all group given EPB. This means that the administration of pasak bumi extract was able to reduce peroxide levels in rats fed high fat and kalori. In the P1 group, the peroxide level was lower than K1, so it can be said that the dose of 7.5 mg/kgBW was able to reduce the peroxide level and this dose was not significantly different from P2 and P3 with higher doses. The group of rats given EPB of 30 mg/kgBW (P4) showed the lowest levels of peroxide and significantly different from P1 and P2, but not significantly different from P3 (dose of 22.5 mg/kgBW). Thus, it can be said that the EPB dose of 7.5 mg/kgBW proved effective.

### Catalase enzyme activity

the K0 group (normal mice) had the highest catalase enzyme activity compared to other groups. Meanwhile, the P4 group that was given EPB at a dose of 30 mg/kgBW showed the highest catalase enzyme activity compared to the other groups given EP. The results of the normality statistical test showed that the data distribution was not normal, so it was continued with the Kruskal-Wallis test with a significance level of 95% to see the effect of EPB administration on catalase activity in the treatment group. The Kruskal-Wallis test showed p value = 0.000 which means that there were significant differences in all treatment groups. This indicates that there is an effect of EPB administration on the catalase enzyme activity of rats.

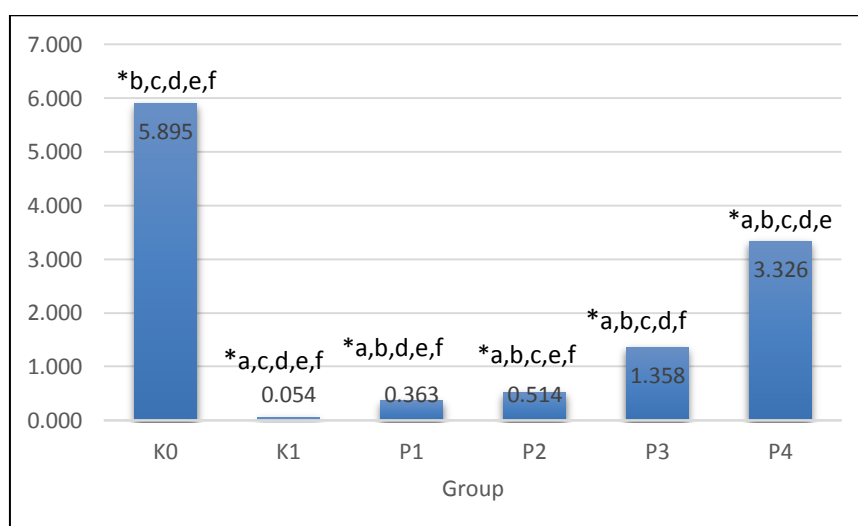


Fig 3. Catalase enzyme activity of *Rattus norvegicus* after administration of *Eurycoma longifolia* Jack, extract ethanol 70%. Note K0=normal +placebo; K1 = high fat and kalori feed +placebo; P1= high fat and kalori feed + EPB 7.5 mg/kgBW; P2= high fat and kalori feed +EPB 15 mg/kgBW; P3= high fat and kalori feed +22.5 mg/kgBW; P4 = high fat and kalori feed +30 mg/kgBW., a: there are significant differences compared with K0 group (p < 0.05); b: there are significant differences compared with K1 group (p < 0.05); c: there are significant differences compared with P1 group (p < 0.05); d: there are significant differences compared with P2 group (p < 0.05); e: there are significant differences compared with P3 group (p < 0.05); f: there are significant differences compared with P4 group (p < 0.05);

The K1 group was significantly different from each of the other groups. This indicates that the administration of EPB with various doses has an effect on the catalase enzyme activity of rats fed the high fat and kalori. Fig. 3 the K1 (high fat and kalori feed +placebo) showed the lowest catalase activity, while the group given EPB dose of 30 mg/kgBW showed the highest catalase activity compared to the other dose groups significantly. Catalase activity increased in proportion to the increase in the dose of EPB administered.

### MDA level

Fig.4 the MDA levels in the normal control group (K0) were the lowest compared to other groups, while the K1(high fat and kalori feed +placebo) showed the highest MDA levels. MDA levels decreased with increasing dose of EPB. To show there is a significant difference between groups, the Kruskal Wallis statistical test was performed because the data were not normally distributed. The results of the Kruskal-Wallis test obtained a significant value of p = 0.012. This means that there is an effect of giving EPB on MDA levels.

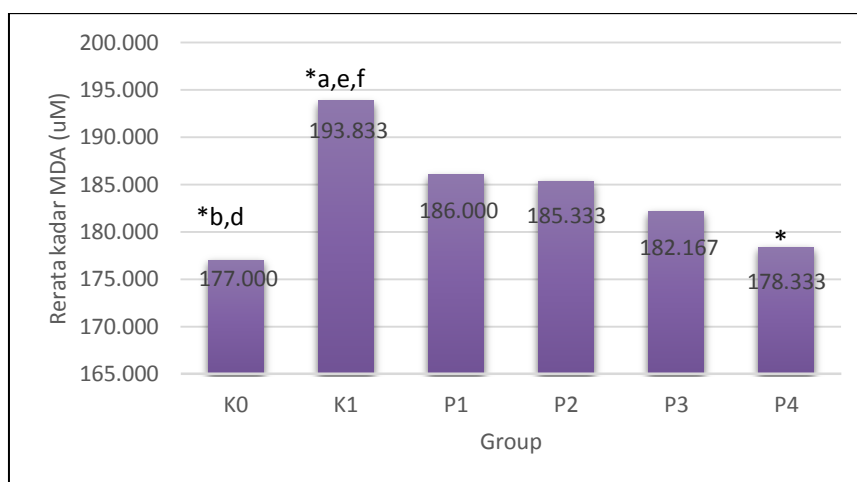


Fig 4. MDA levels of *Rattus novergicus* after administration of *Eurycoma longifolia* Jack, extract ethanol 70%. Note K0=normal +placebo; K1 = high fat and kalori feed +placebo; P1= high fat and kalori feed + EPB 7.5 mg/kgBW; P2= high fat and kalori feed +EPB 15 mg/kgBW; P3= high fat and kalori feed +22.5 mg/kgBW; P4 = high fat and kalori feed +30 mg/kgBW., a: there are significant differences compared with K0 group ( $p < 0.05$ ); b there are significant differences compared with K1 group ( $p < 0.05$ ); d: there are significant differences compared with P2 group ( $p < 0.05$ ); e: there are significant differences compared with P3 group ( $p < 0.05$ ); f: there are significant differences compared with P4 group ( $p < 0.05$ );

MDA levels in the normal rat group (K0) were significantly different from K1. This has proven that the administration of high fat and kalori diet can increase MDA levels, and after being given EPB there is a decrease in MDA levels in groups P1, P2, P3 and P4. MDA levels in the P3 and P4 groups were lower than those in the P2 group and were not significantly different from the control group (K0). Meanwhile in the P1 and P2 groups, although they had lower MDA levels than K1, they were not significantly different. This indicates that the doses of 7.5 mg/kgBW and 15 mg/kgBW have not been able to reduce MDA levels in rats fed the TKTL diet. However, in mice that were given a larger dose of EPB, namely 22.5 mg/kgBW (P3) and 30 mg/kgBW (P4), the MDA levels were significantly lower than K1. Meanwhile, the MDA levels in the P3 group were not significantly different from that in the P4 group. This proves that the EPB dose of 22.5 mg/kgBW has been able to reduce the MDA levels of rats fed the TKTL diet as well as the dose of 30 mg/kgBW.

#### IV. Discussion

Obesity is a nutritional disorder, characterized by abnormal or excessive fat accumulation, as a result of adipocyte hypertrophy and/or hyperplasia. It represents a serious risk to health, as it increases the likelihood of several pathologies (including metabolic syndrome, Type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, and cancer).<sup>12</sup> During obesity the adipose tissue undergoes pathological change due to absorption of fat viz. inflammation, and oxidative stress and this in turn enhance to secretion of adipokines and affect the peripheral tissues that produce ROS, which stimulates oxidative stress and inflammatory response.<sup>13</sup>

The study of Gusti et al 2021, found that in obese people SOD levels were lower than non-obese, this result was in accordance with the research of Rusdiana et al 2017 that the SOD levels of obese people with metabolic syndrome were lower than obese people without metabolic syndrome. The study of Sabah et al 2019 found that obese children had higher peroxidase levels than children with normal weight.<sup>14,15</sup>

Based on the research of Triawanti et al (2020) obtained 70% ethanol extract of pasak bumi that grows in Kalimantan contains 8.73% saponin compounds, 14.47% alkaloids, flavonoids 21.5 mg/mL, steroids 42.28 mg/mL, terpenoids 244.3 mg/mL, and tannin 2.33 mg/mL. These compounds, especially flavonoids, have potential as antioxidants.<sup>9</sup> In Figure 5.1 it can be seen that EPB administration can increase SOD levels compared to placebo, EPB doses of 7.5-22.5 mg can significantly increase SOD levels in treated mice compared to the control group, with a maximum dose of 22.5 mg/kg BW of EPB. Superoxide dismutase (SOD) is an antioxidant that works to protect cells against free radicals and ROS, so that EPB administration has the potential to increase SOD levels in obese.

In this study, it was found that the increase in peroxide levels was higher in rats that received the high fat and kalori diet without the addition of EPB, while the rats with the high fat and kalori diet and EPB diets also experienced an increase in peroxide although not as high as in the group without EPB. Under normal conditions the body has a natural protective mechanism against free radical damage with a balance between oxidants and antioxidant mechanisms including the presence of natural antioxidant enzymes and free radical scavengers mechanisms as well as the results of catalase activity in this study. where high catalase levels were found at a dose of 30 mg/kg BW of EPB although this result was still below the group without EPB administration.

Malondialdehyde (MDA) is a product of the lipid peroxidation reaction. Fig.4 shows that the MDA levels in the group of obese rats given placebo (P1) were higher than in other groups. EPB dose of 22.5 mg/kgBW can reduce MDA levels.

Noticeably, in obese individuals, oxidative stress is so closely interlinked with inflammation as to trigger a vicious circle: oxidants activate specific redox-sensitive transcription factors [including nuclear factor- $\kappa$ B (NF- $\kappa$ B) and activator protein-1 (AP-1)], which drive the expression of pro-inflammatory cytokines; these mediators, in turn, enhance production of reactive oxygen species (ROS), thus contributing to the onset and maintenance of oxidative stress.<sup>12</sup> The increase in free radicals or reactive oxygen species must be balanced by an increase in the synthesis of enzymatic antioxidants. But often this is not enough to be overcome only with endogenous antioxidants, so exogenous antioxidants are needed. The lack of endogenous antioxidant defenses in the body can be overcome by administering exogenous antioxidants, so that the balance of reactive oxygen species and antioxidants can be reestablished, preventing damage from occurring.

## V. Conclusion

EPB doses of 7.5-22.5 mg can significantly increase SOD levels in treated mice compared to the control group, with a maximum dose of 22.5 mg/kg BW of EPB. EPB can reduce MDA and peroxide level in obese rats.

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