The beneficial effect of *Parkia speciosa*'s empty pods extract on lipid profile of *jelantah* treated wistar white rats

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Abstract. *Parkia speciosa* have many potential properties, as source of antioxidant to combat free radicals, including by dint of *jelantah* exposure. Its flavonoid content is suspected as an antihiperlipidemia so it can be used as natural treatment to control the lipid profile. This study aims to examine the beneficial effects of the ethanolic extract of *P. speciosa*'s empty pods (EEPE) on the cholesterol, LDL, HDL, and triglycerides levels in *jelantah* exposed Wistar rats. The rats were divided into four groups, group I as negative control with no jelantah and EEPE treatment, group II with 1 mL *jelantah* (118 meq/kg) and 100 mg/kg body weight of EEPE, group III with 1 mL jelantah (118 meq/kg) and 200 mg/kg body weight of EEPE, and group IV as positive control with only jelantah treatment. This study showed that the higher concentration of EEPE able to reduce the cholesterol, LDL, and triglyceride levels also increase the HDL level more than the lower one. However, the treatment of EEPE in group I and II cannot regenerate the lipid profiles in group I as negative control.

1 Introduction

One of Indonesian habits is consuming kind of fried foods especially which are fried with repeated used of palm oil, known as *jelantah*. These habit is one of dyslipidemia trigger factors in Indonesia [1]. The *jelantah* is repeatedly heated palm oil until the color has changed which can trigger some harmful compounds in the body due to its high amount of saturated fatty acids. There is a recommendation to decrease the intake of saturated fatty acid (less than 10%) of total energy intake so the metabolic diseases could be avoided [2]. Using the same palm oil for up to ten hours leads to the formation of tert-butyl-hydroquinone as main oxidation product which has the cytotoxic effect. The *jelantah* able to increase radicals level in the body and provides enhancement of oxidative stress [3].

The use of bioactive compounds from plants as an alternative solution instead of chemical drugs has recently expanded to combat the oxidative stress since the use of jelantah in Indonesian fried food has increasing. One of Indonesian's local plants as source of antioxidant is stinky beans, known as petai or *P. speciosa*. This plant has been reported as antioxidant, anti-inflammatory, antihypertensive, and possess hypoglycemic properties [4].

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Petai is abundantly found in some Southeast Asian countries, like Indonesia, Malaysia, Philippines, and Thailand. It is also found in Africa, India, and Madagascar [5]. It has several bioactive compounds like saponins, tannins, alkaloids, flavonoids, phenolics, terpenoids, xanthoproteins, and glycosides [6].

In this study, the empty pods of *P. speciosa*, which are usually just thrown away, were assumed as an alternative herbal medicine to treat dyslipidemia condition. It is an abnormal condition of metabolism of lipoprotein, such as abnormality of total cholesterol, LDL (low-density lipoprotein), HDL (high-density lipoprotein), and triglyceride status [1]. The *P. speciosa* empty pods usually ended up in the trash bin because Indonesian only consumes its seeds. Whereas, the empty pods have such a great level of antioxidant, antiangiogenic, and antidiabetic functions. The objective of this study is to analyze the potential effect of the ethanolic extract of *P. speciosa* empty pods (EEPE) to lower total cholesterol, LDL, and triglyceride level, and on the other hand increase the HDL level so the dyslipidemia condition can be controlled.

2 Materials and methods

2.1 Extraction of *P. speciosa*'s empty pods

The extraction process was based on previous research [1] [3] with maceration method. The empty pods were separated from the seeds then be sliced into smaller pieces. The small pieces of empty pods then dried for 48 hours in dryer cabinet at 50°C. The dried empty pods were mashed and sieved in 80 mesh sifter so the powders were remaining. A twenty gram of powdered empty pods were macerated with 500 mL of ethanol (Merck) for 24 hours then re-macerated three times. The filtrate was collected then evaporated with vacuum rotary evaporator (DLab) at 45°C until all solvent was evaporated.

2.2 Experimental design for in vivo test

In this study, we used Wistar white rats (Rattus norvegicus) as animal models. The ethical clearance ID number for animal models is 606/PL17.4/PG/2023 which obtained from State Polytechnic of Jember, East Java, Indonesia. This experiment was performed with a control group post-test design. The Wistar white rats were grouped into four, with five rats in each group. The group one (G1) is negative control, with no treatment; group two (G2) was treated with 1 mL of jelantah (118 meq/kg of body weight) and 100 mg/L of EEPE; group three (G3) was treated with 1 mL jelantah (118 meq/kg of body weight) and 200 mg/L of EEPE; and group four (G4) is positive control was only treated with 1 mL jelantah (118 meq/kg of body weight) without EEPE. The Wistar white rats were adapted for four days in the laboratory and fed with 20 g of AIN93M standard feed per day during experimental period. All four groups were treated for twenty eight days with jelantah and also EEPE (per oral) in accordance with the experimental design. After twenty eight days of the treatment, the blood serum was collected from its orbital sinus for further analysis.

2.3 Measurement of total cholesterol (TCL), LDL, HDL, and triglycerides level (TL)

The blood serum from the all four groups of animal models were used to measure the TCL, LDL, HDL, and TL. The all four measurements were performed at Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. The kit used in this study were cholesterol quantitation kit (Sigma Aldrich), HDL and LDL/VLDL quantitation

kit (Sigma Aldrich), and triglycerides quantification colorimetric/fluorometric kit (Sigma Aldrich).

2.4 Statistical data analysis

The analysis of variance was conducted using one way ANOVA with SPSS 25 version. The significant differences were then determined with Duncan's multiple range test. The statistically significant values were considered when p-value was less than 0.05.

3 Results and discussions

3.1 The extraction yields

The global extraction yield is one of major interest in plant extraction because the yield assesses the performance of extraction method [7]. The extraction of 20 g of *P. speciosa* empty pods produce 3.27 g of EEPE (16.35 % of yield) [8]. This yield was lower than methanolic and aqueous extract which were 22.80 % and 24.95 %, respectively, but higher than n-hexan extract which was 15.25 % [4]. Ethanol was used as solvent in this study because it is a universal solvent which has an ability to binds or dissolves compounds extracted from natural materials, either polar, semi-polar, or non-polar [9].

3.2 The total cholesterol level (TCL)

The total cholesterol level is one of important markers for coronary heart disease associated with lipid profile in the body. Hypercholesterolemia occurs when the total cholesterol level (TCL) in the bloodstream is elevated [10]. The enhancement of TCL changes the membrane lipids to form reactive oxygen species (ROS) leads to DNA, proteins, and membranes damage [11].

The G4 as positive control with only jelantah treatment and no EEPE in this study showed the highest TCL in 184.35 mg/dL than other three groups. It was indicated that jelantah is potentially increase the TCL in the bloodstream leads to hypercholesterolemia. On the other hand, the G1 as negative control with no jelantah or EEPE treatment showed the lowest TCL in 98.26 mg/dL. The G2 and G3 showed 148.99 and 116.81 mg/dL of TCL, respectively (Table 1). The TCL of G3 is lower than G2 because of higher concentration of EEPE which were given. This result indicated that the EEPE have ability to reduce TCL caused by jelantah treatment in Wistar rats. The higher EEPE concentration, the lower TCL, and vice versa. Based on this result, it is assumed that the EEPE can be an alternative to control TCL in the bloodstream.

Table 1. The total cholesterol level (TCL) of Wistar white rats after twenty eight days of *jelantah* and EEPE treatment.

Group	Total cholesterol level (mg/dL)
G1	$98.26^{a} \pm 2.33$
G2	$148.99^{c} \pm 3.79$
G3	$116.81^{b} \pm 2.68$
G4	$184.35^{d} \pm 2.64$

*Different letters in the superscript within column indicate the significant difference ($p < 0.05$)

The increase in TCL was caused by induction of jelantah in the experimental treatment. However, the TCL was decrease due to EEPE treatment. It was seen in G2 and G3 which have lower TCL than G4 which had no EEPE treatment. The ability of EEPE to reduce TCL is predicted as a correlation with its flavonoids content. Flavonoids in the EEPE is well known as antioxidant that can neutralizes oxygen radicals and fatty acids, effectively [1].

3.3 The low-density lipoprotein (LDL) level

The LDL level can be increased due to high exposure of saturated fatty acids. The high LDL could increase the risk of coronary heart disease [11]. The LDL level also associated with the risk of atherosclerosis which is play the important physiological role in the delivery of cholesterol to the peripheral tissues [12]. The high level of LDL triggers accumulation of the cholesterol in the blood vessels which causes formation of plaque in the walls of blood vessels [13].

Table 2 showed that G4 has the highest LDL level in 80.75 mg/dL because this group was only treated with 118 meq/kg of jelantah. On the other hand, G1 showed the lowest LDL level in 28.83 mg/dL because this group is a negative control with no treatment at all. For G2 and G3, the data showed that G3 has lower LDL level than G2, 45.58 mg/dL and 60.23 mg/dL, respectively. The data showed that EEPE treatment can reduce the LDL level in Wistar rats with jelantah exposure. The higher EEPE concentration leads to lower LDL level and the lower EEPE concentration leads to higher LDL level as seen at G3 and G2.

The increased level of LDL in the blood triggers the emergence of atherosclerosis. This disease has multifactor reasons, including hypercholesterolemia, hypertension, diabetes mellitus, obesity, and also environmental factors [13]. This LDL is major lipoprotein which assists lipid transfer in the extracellular fluid then leads to atherosclerosis by attracting the macrophages into blood arterial walls. This LDL is known as bad cholesterol and associated with occurrence of stroke and heart attack [14].

Table 2. The low-density lipoprotein (LDL) level of Wistar rats after twenty eight days of *jelantah* and EEPE treatment.

Group	LDL level (mg/dL)
G1	$28.83^{a} \pm 1.95$
G2	$60.23^{c} \pm 1.80$
G3	$45.58^{b} \pm 2.30$
G4	$80.75^{d} \pm 1.92$

*Different letters in the superscript within column indicate the significant difference ($p < 0.05$)

3.4 The high-density lipoprotein (HDL) level

The HDL level is negatively correlated with the atherogenic index [15]. High-density lipoprotein is the smallest yet most dense lipoprotein that contains the highest proportion of the protein to the lipids. In the health condition, about 30 % of cholesterol in the blood was carried of high density lipoprotein which is known as the good cholesterol. The individual with higher HDL tent to have fewer cardiovascular problems. The high HDL levels have been correlated with better cardiovascular condition than the lower one [14].

In this study, the G4 as positive control showed the lowest HDL level in 19.75 mg/dL because it was exposed with jelantah without EEPE. The highest HDL level is 78.51 mg/dL in G1 as negative control. The G3 with higher EEPE concentration showed higher HDL level than G2 with lower EEPE concentration, that are 65.56 mg/dL and 53.28 mg/dL, respectively.

However, HDL level of G1 still the highest compared to G2 and G3. It indicates that even though HDL level can be increased with EEPE treatment, but it will never as high as HDL level without jelantah exposure (Table 3).

Table 3. The high-density lipoprotein (HDL) level of Wistar rats after twenty-eight days of *jelantah* and EEPE treatment.

Group	HDL level (mg/dL)
G1	$78.51^{d} \pm 1.72$
G2	$53.28^{b} \pm 3.59$
G3	$65.56^{c} \pm 2.42$
G4	$19.75^{a} \pm 1.60$

*Different letters in the superscript within column indicate the significant difference ($p < 0.05$)

Antioxidant activity of flavonoid content in EEPE can increase the HDL level. The TCL and HDL level affect the atherogenic index value. The reduction of TCL and enhancement of HDL level lower the atherogenic index value, so it is also decreasing the risk of atherosclerosis [16].

3.5 The triglycerides level (TL)

The consumption of saturated fatty acids from some fried food in high amounts is one of the major factor leads to enhancement of cholesterol and triglycerides level in plasma [13]. Triglycerides are the esters which derived from glycerol and three fatty acids. It is playing an important role in the metabolism as an energy sources and also transporters of dietary fats. Triglycerides are the major component of the very low-density lipoprotein (VLDL) and the chylomicrons [12].

The result of this study showed that the TL trend was similar with TCL and LDL. The highest TL is in G4 that is 148.94 mg/dL and the lowest is G1 in 85.39 mg/dL. The G2 and G3 is in between G1 and G4, which are 116.60 and 102.70 mg/dL, respectively. The TL of G3 is lower than G2 because of higher EEPE concentration. It indicates that EEPE has ability to reduce the TL even though it still higher than G1 which was not exposed by jelantah at all (Table 4).

Table 4. The triglycerides level (TL) of Wistar rats after twenty eight days of *jelantah* and EEPE treatment.

Group	TL level (mg/dL)
G1	$85.39^{a} \pm 2.49$
G2	$116.60^{c} \pm 3.97$
G3	$102.70^{b} \pm 3.07$
G4	$148.94^{d} \pm 5.74$

*Different letters in the superscript within column indicate the significant difference ($p < 0.05$)

The combination of high TCL, LDL, TL, and HDL level is physiologically possessing an important role in the body. High level of TCL, LDL, and TL lead to major risk of some consequences of atherosclerosis such as cerebral infarction (stroke), sudden cardiac death, chronic ischemic heart disease, peripheral vascular disease, ischemic encephalopathy, aortic ancurysms, and myocardial infarction (heart attack) [12].

4 Conclusion

The ethanolic extract of *P. speciosa* empty pods is proven as one of herbal alternative to reduce the hyperlipidemia by reduce the TCL (total cholesterol), LDL (low-density lipoprotein), and TL (triglycerides level). This extract was also proven to increase the high-density lipoprotein (HDL) level which further decrease the risk of atherosclerosis. The ethanolic extract of *P. speciosa* empty pods has an ability to prevent cardiac and vascular disease so it also reduces the risk of stroke and heart attack which is one of biggest world killer diseases.

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