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Phaleria macrocarpa (Scheff.) Boerl. Pulp Extract Increases the Sperm Characteristics in *Rattus norvegicus*

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Abstract

Herbal remedy is a type of alternative medicine that originates from plants and have been around for centuries. This study to analyze *Phaleria macrocarpa* L. pulp extract (PMPE) on male fertility by assesing its effect on sperm characteristic including sperm count, motility, viability and membrane integrity.Twenty four males adult of *Rattus norvegicus* were seperated into four groups. The treatment of three groups were done orally, once a day with 0.25 ml supplemented for four weeks either with PMPE at doses 7.5 mg/kg (P1), 15 mg/kg (P2), 30 mg/kg (P4) and CMCNa 0.5% (P0). PMPE significantly increases the percentage of sperm characteristic.

Key words: *Phaleria macrocarpa*, motility, viability, membrane integrity

The serious problems that we face today is infertility and about 8-12% of couples suffer from across the globe. Of all infertility cases, approximately 40-50% is due to male factor infertility and as many as 2% of all men exhibit suboptimal sperm parameters (Kumar *et al*, 2015). Among the numerous causes of male

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infertility, oxidative stress (OS) can cause the cellular damage by free radicals that can ultimately impact sperm quality. Free radicals were reported to attack cell metabolites directly, resulting in decreased of ATP levels in sperm cell movement and function (Agarwal *et al*, 2014).

Medicinal plant offer some advantages for some reason, have been approved globally (Koduru *et al*, 2007). *Phaleria macrocarpa* L.(PM) is one of the herbal medicine that reported has flavonoids, phenolics and antioxidant properties (Altaf *et al*, 2013). Some research have published that flavonoids can increase the sperm quality by protecting it from free radicals (Adewoyin *et al*, 2017). PM was claimed to increase fertility of men but scientific data is not enough yet to prove it. Therefore, the aim of this research is to analyze PMPE on the sperm's quality on *Rattus nirvegicus*.

Materials and Methods

Phaleria macrocarpa L. (PM) pulp were collected from Sidoarjo, East Java, Indonesia. 700 g PM pulp slice were cut into small pieces. Slice of PM were dried in a room until 5 days then dried again in oven at 50 ◦ C then powdered using mechani-cal blender and passed through the coarse sieve (0.2 mm).The PM powder was macerated with ethanol 96% for 72 h at 37 °C. The extract was evaporated in water bath at 60°C. The residue was stored in a refrigerator at -4°C.

Twenty four *Rattus norvegicus* of two months of age and about 250-300 g body weight were used. They were placed in biochemistry laboratory, Faculty of Medicine, Universitas Airlangga at 28-33 ◦ C. The rats were free from disease and deformities. Before treatments they were adapted for a week and fed with pellet and given water *ad libitium*. This study used randomized experimental design with three various doses of PM (7,5 mg/kg (P1), 15 mg/kg (P2). 30 mg/kg (P3)) and control given CMCNa 0,5% (P0). Cervical dislocation was used to kill the rats and the cauda epididymis were removed quickly. The cauda epididymis were cut longi-tudinally with forceps and scissors. Two petri dishes were filled with phosphate buffer saline (PBS) to collect the sperm by mincing the cauda epididymis.

The number and the motility of sperm was found using a haemocytometer in 20 µL solution of the sperm dropped on a cover slip. Using light microscopy, it was counted in 4x4 squares (horizontally or vertically), at 400x magnification. For the viability test, petri dish was prepared with 0,1 M PBS. 20 µL of sperm solution were dropped then mixed with 30 µL of eosin-negrosin and smear with the glass side tip and air-dried.Using light microscopy, the sperm were counted at 400x magnification. While the hos test, 100 µL of sperm were mixed in 900 µL of 60 mOsmol fructose solution. 20 µL were dropped to make smear and then incubated at 37◦C for 30 min. Using fluorescence microscopy, the sperms were counted at 400x magnification.

The data was analyzed by using SPSS 21 (SPSS Inc., Chicago, IL), employing one-way analysis of variant (ANOVA) ($p<0.05$) and followed by Tukey HSD. Tabulated data are presented as the mean ± standard deviation.

Results and Discussion

The sperm count was campared to the total of the sperm in various concentarions. The highest amount of sperm count was found at P2 (940 million cells/ml), P1 was 810 million cells/ml and when it come to P3 the amount of sperm decreases (710 million cells/ml) compared to P2. The percentage showed no difference between the P0 and P3 significantly, while sperm count in P1 and P2 was significanly different from P0.

The result in fig 1 showed that the highest motility value is found at P2 with mean score of 71.7% compared with P1 of 67.6%.The

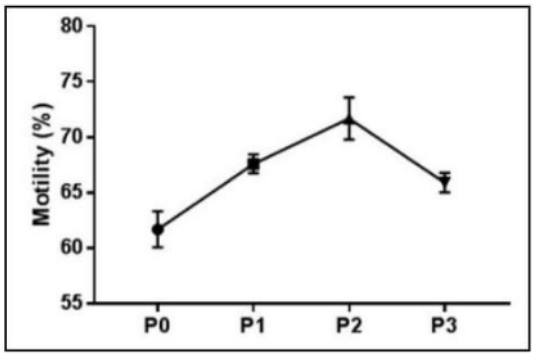

Fig 1. Effect of the PMPE on sperm motility of *Rattus norvegicus*.

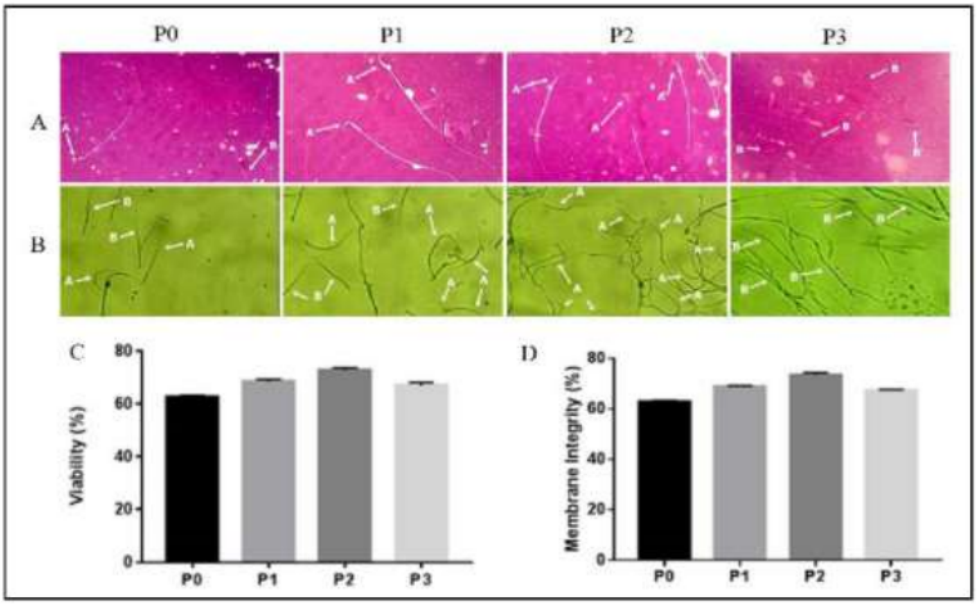

Fig 2. Effect of the PMPE on rat sperm, the untreated group (P0), following group treated 7.5 mg/kg (P1), 15 mg/kg (P2), 30 mg/ kg (P3). A) The viablity of rat sperm with eosin-negrosin staining.The viable sperm showed colourless head (A) and the dead sperm showed red head (B). B) The membrane integrity of rat sperm with hypo osmotic swelling test. Good sperm with pres-ence of coiled tail (A) and damaged sperm with absence of coiled tail (B). The bars (C,D) represent the mean ± SD of viablity and membrane integritypercentage.

lowest value showed at P0 of 61.7%, while the concentration of P3 shows decreasing sperm value of 65.9% comapred with P2. This increase in sperm motility could be due to the protective effect of PM administration (P1, P2) and increase in total antioxidants capacity.

The result in figure 2(A,C) showed that the highest value is found at P2 with mean score of 72.8% compared to P1 of 68.4% .The lowest value showed at P0 of 62.6%, while P3 showed decreasing sperm value of 67% comapred to P2. This increase in sperm motility could be due to the protective effect of PM administration (P1, P2) and increase in total antioxidants capacity. The antioxidants such as flavonoids can provide the protection of the plasma membrane and reduce the excessive amount of ROS in rat sperm (Khaki *et al*, 2011). Antioxidant are subtances that can neutralize free radicals in the body and against damage due to free radicals to the cells that have normal conditions. Free radicals were neutralized by antioxidant with supplementing electron deficiencies of chain reactions then block the occurrence of chain reaction from free radicals formation and due to that the oxidative stress will not perform out of the body (Phanien-dra *et al*, 2015).

The percentage of Hos test showed that the highest value is found at P2 and has the mean score of 73.4% compared to P1 of 68.8%. The lowest value showed at P0 of 62.8% and while P3 showed decreasing sperm value of 67.2% comapred to P2. The integrity of membrane of the rat sperm is seen in the fig 2 (B,D). Sperm is wrapped by membrane which serves as a protection against environmental changes. If the sperm is exposed to the hypo osmotic medium, the sperm will swell or curve because the cell membrane is semi permeable and functions normally (Talwar and Hayat-nagarkar, 2015). Meanwhile, P3 have trend in decreasing in all group. Decreasing sperm membrane integrity comes from excess antioxidants. Antioxidants will function if there are pro-oxidants compounds in the body. When the dose of anti-oxidants and pro-oxidants are

not balanced, the body will form pro-oxidants to balance the way then damage the integrity of the sperm plasma membrane and interfere with the function of the mitochondria to produce ATP for movement of sperm (Choudhary *et al*, 2010) then DNA damage caused by oxidative stress that increases ROS formation, which can damage DNA fragmentation resulting in apoptosis. This results in increase of dead sperm (González-Marin *et al*, 2012).

Antioxidant activities of PMPE were due to the presence of flavonoids that can increase the viability, motility and membrane integrity of the sperm in the right doses (Jamalan *et al*, 2016).

Summary

In conclution, this study provides the evidence that PMPE can act as antioxidant, enchanches the quality of the rats sperm including motility, viability and membrane integrity. PMPE have cytotoxic effect if it was not given in the right doses. We suggest that PMPE could be one of the herbal source for studying on reproduction stage.

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