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Antifertility Effect of N-hexane, Ethyl Acetate and Water Fractions from *Carica papaya* Leaves on Sperm Motility in Male Wistar Rats

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***Abstract*-Objective : The aim of this study was to investigate the antifertility effect of N-hexane, ethyl acetate, and water fractions from *C. papaya* leaves on sperm motility in male Wistar rats. Method : Twenty four rats used in this study. Rats were devided into 4 groups : normal group and *Carica papaya* fraction group (N-hexane; ethyl acetate; and water). Rats were given *Carica papaya* fractions at dose 0.4 g/ Kg BW rat/day for 20 days). *Carica papaya* leaves were extracted by ethanol and did fractionation by liquid-liquid methods. Results and Discussion : The results of this study were N-hexane, ethyl acetate and water fractions significantly (P<0.05) produced decreased in sperm motility compered with normal group. The decresed *Carica papaya* fractions (N-hexane, ethyl acetate and water) were 33 %, 65%, and 75% respectively. Conclusion : *Carica papaya* fractions (N-hexane, ethyl acetate and water) have antifertility effect on sperm motility.**

Keywords: antifertility,* Carica papaya*, sperm motility, N-hexane, ethyl acetate, water fraction

I. INTRODUCTION

Based on the Ministry of Health and Information Center data in 2017, the population in Indonesia were 261,890,872 people that divided into 131,579,184 male populations and 130,311,688 female populations. Population growth in Indonesia by 2017 were 3.19 million per year. High population growth will result an increase in population density lead to uneven effects of welfare, such as increasing poverty due to decreasing employment opportunities and decreasing community health status. One of the efforts made by the Government to regulate the rate of population growth in Indonesia is the establishment of a Keluarga Berencana (KB) or family planning program [1]. Keluarga Berencana (KB) is a programme from Indonesia government to control the growth of populations. KB has a goal to increase the quality of life in family by forming qualified small family [2], to regulate the birth of children, both the number or the distance between births of children to create a good quality family and achieve physical and spiritual well-being [1].

Since the family planning program has been established until now, the attention and implementation of the program in Indonesia tends to be more focused on women. This creates a stigma in the community that family planning is a matter and responsibility of women. In addition, compared to female contraceptive methods, male contraceptive methods have fewer types and relatively rarely used [3,4]. Until now, the available modern male contraceptive methods are vasectomy and condom. While the traditional male contraceptive method is interrupted intercouse. Globally, the rate of vasectomy and condom use is 8.9% of total contraceptive use. Based on a survey conducted by the National Population and Family Planning Agency in 2017, the rate of vasectomy use was 0.53%, condom use was 1.22%, and interrupted intercourse was 1.32%. This showed that men's participation in contraceptive use was still very low [1,5]. The use of condom and interrupted intercourse cause perceptions of psychological problems such as reducing sexual pleasure. The use of vasectomy cause perceptions of side effects and impotence problem make the participation of men in family planning programs to be very limited [3].

The World Health Organization established a Task Force program to examine the potential of traditional herbs or plants to develop traditional herbs as an alternative and new method of contraception to control birth rates. Traditional herbs that have antifertility effects must be safe, effective, reversible, do not interfere with sexual activity, and can be accepted by the community. Indonesia is a tropical country that has a high level of biodiversity in all its regions. This high biodiversity makes Indonesia have various types of plants that have potential as traditional herbs medicine. Herbs can be used as an alternative treatment and prevention of various diseases or certain health conditions with minimum side effects and prevent side effects from pharmacological drugs. Various herbs in Indonesia have also been shown to have the potential antifertility effects in men [4-7].

Indonesian plants or herbs have male antifertility effect, such as papaya seeds (*C. papaya*); fruit and seeds of bitter melon (*M. charantia*); betel leaf (*P. betle*); flowers, leaves and roots of hibiscus (*H. rosa*); turmeric (*C. domestica*), betel nut (*A. catechu*); and sambiloto leaf (*A. paniculata*). These herbs contain several phytochemical substances such as flavonoids, saponins, tannins, steroids, triterpenoids, alkaloids, terpenes, estradiol (E2) and progesterone (P4) which cause disorders in both the anatomy and physiology of the male reproductive organs such as GnRH suppressant and make the testosterone drop level hormones. Steroid, triterpenoids, alkaloids, estradiol (E2) and progesterone (P4) are cytotoxic and anti-androgenic [6]. This will cause disruption of spermatogenesis results in decreased viability, motility, and morphology of sperm [7].

Carica papaya commonly called papaya, a tropical and subtropical plant of the family Caricaceae [5,8,9,16]. In reproduction, various extracts of *C. papaya* have been shown

to have antifertility activity in male [8-15]. Papaya seeds have been shown to influence the fertility of male mice by influencing sperm quality such as reducing the number spermatogonia A [10], decreasing sperm motility from 47% [10], decreases sperm viability [10,11], and increases sperm abnormality ($P < 0.01$) [10]. Papaya roots have been shown to influence sperm quality. The fractions (petroleum ether, chloroform, ethyl acetate and methanol) significantly ($P < 0.01$) produced decreases in sperm counts and increased the percentage of defective sperm cells [13]. Aqueous extract of *C. papaya* leaf caused reduction in mean values of andrological parameters as a result of lesion of the seminiferous tubule epithelium [14]. *Carica papaya* methanol leaf extract (CPMLE) significantly ($p<0.01$) produced dose dependent decreases in sperm counts and increased the percentage of defective sperm cells [15]. Following the above facts about the activity of *C. papaya*, it became necessary to undertake this research.

II. MATERIAL AND METHOD

Plant material

The study material was Papaya Leaf, which was collected from the Rajadesa , Ciamis Regency, West Java. The raw material was confirmed its identity by determinated at the Plant Taxonomy Laboratory, Department of Biology FMIPA Padjajaran University, Bandung.

Experimental animals

Adult male Wistar rats weighing between 200-250 g were used for the study. They were kept in the animal house of Food and agriculture departement , Bandung. The animals were provided with pelleted diet and water *ad libitum*. They were allowed a one-week acclimatization period before the experimental session. All the experimental protocols were met with the approval of Padjadjaran university research ethics committee.

Plant extraction

1.550 grams of the dried pulverized leaves were extracted with 70% ethanol in water for 72 h with intermittent shaking every 2 h. This was filtered with Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator at 40°C.

Fractions

N-Hexane fractions

As much as 25 grams of ethanol extract was dissolved in 250 mL water then fractionated using n-hexane in a ratio of 1: 1, shaken and then allowed to stand until two layers formed. The n-hexane layer is separated and new n-hexane is added to the ethanol-water layer. The process of adding solvents was repeated 3 times. The obtained fraction is then concentrated using a Rotary evavorator.

Ethyl acetate fractions

The insoluble part of n-hexane is fractionated with ethyl acetate, shaken and allowed to stand until two layers are formed. The ethyl acetate layer is separated and new ethyl acetate is added to the n-hexane layer. The process of adding solvents was repeated 3 times. The obtained fraction is then concentrated using a Rotary evavorator.

Water fractions

The insoluble part of ethyl acetate, fractionated with water, is shaken and then allowed to stand until two layers are formed. The water layer is separated and new water added to the ethyl acetate layer. The process of adding solvents was repeated 3 times. The obtained fraction is then concentrated using a Rotary evavorator

Phytochemical screening

Freshly prepared ethanolic extract was subjected to quantitative analytical tests for the detection of various chemical constituents like saponins, flavonoids, tannins and polifenols, steroids and triterpenoids, quinons, alkaloids, mono and seskuiterpenoids [18]. Phytochemical screening is carried out on simplicia, extracts and fractions.

Treatment

Twenty four rats used in this study. Rats were devided into 4 groups : normal group and *Carica papaya* fraction group (N-hexane; ethyl acetate; and water). Rats were given *Carica papaya* fractions at dose 0.4 g/ Kg BW rat/day for 20 days).

Sperm motility

The sperm motility is measured by looking at the sperm velocity in the count chamber neubaure. One drop of sperm suspension in a 0.9% NaCl solution dripped on the count chamber was then observed under a 400 times magnification microscope. The number of motile sperm was quickly calculated based on the WHO criteria, namely; Progressive motility (PR): the spermatozoa move actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (NP): all other patterns of motility in the absence of progress, such as swimming in small circles, flagellar strength barely displacing the head, or when only flagellar beats can be observed. Immotility (IM): no movement. Observations made on 200 sperms, then repeated as much as 3 times for one rat and the result is averaged. The sperm motility is expressed in percent units. The percentage of motile sperm count was determined by summing the PR + NP category, divided by the number of categories PR + NP + IM then multiplied by 100%. [19].

Statistical analysis: The data collected were statistically analyzed using one-way Analysis of variance (ANOVA) and Duncan New multiple range *post hoc* test, mean differences at $p<0.05$ were considered significant.

III. RESULTS

Extraction of the plant material

The yield of the ethanol extraction of *Carica papaya* leaves was 12.26%.

Fractionation of Papaya Leaf Ethanol Extract

Solid extract as much as 125 grams was obtained by the yield of n-hexane fraction as much as 21.04%, ethyl acetate fraction as much as 19.88%, while the water fraction produced a greater yield of 57.12%.

Phytochemical screening results of simplisia, extract and fraction

The phytochemical screening result can be seen on Table 1.

TABLE 1. **Phytochemical screening results of *Carica papaya* L. simplisia, extract and fractions**

No	Metabolits	S	E	NH	EA	W
1	Alkaloids	+	+	-	-	+
2	Flavonoids	+	+	+	+	+
3	Tanins dan Poliphenols	+	+	+	+	+
5	Saponins	+	+	-	+	+
6	Monoterpens and seskuiterpens	+	+	+	-	+
7	Steroids dan triterpenoids	+	+	+	+	-
8	Quinons	+	+	+	+	+

+ = presence, − = absence, S : Simplisia, E : Extract, NH : N-hexane, EA : Ethyl acetate, W : Water fraction

Sperm motility activity

Descriptive statistics related to the results of *Carica papaya* fractions with normal group are shown in Figure 1.

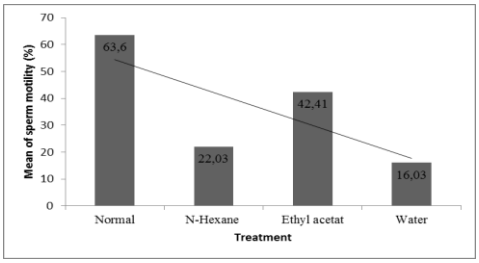

Figure 1. The mean spermatozoa motility (%)

IV. DISCUSSION

The results of this study were N-hexane, ethyl acetate and water fractions significantly (P<0.05) produced decreased in sperm motility compered with normal group. The decresed *Carica papaya* fractions (N-hexane, ethyl acetate and water) were 33 %, 65%, and 75% respectively. *Carica papaya* fractions (N-hexane, ethyl acetate and water) have antifertility effect on sperm motility.

Alkaloids-containing Carica papaya seeds seeds are cytotoxic and anti-androgenic [6,10]. Cytotoxic effects will cause disruption of spermatogenic cell metabolism while the antiandrogenic effect works by press the hypothalamus and the anterior pituitary that causing gonadotropin-releasing hormone (GnRH) inhibition which results in folliclestimulating hormone (FSH) and Luteinizing-hormone (LH). Low levels of LH will inhibit the secretion of testosterone hormone and low FSH will cause disruption in sertoli cells. Disorders of sertoli cells can cause disruption in the process of spermatogenesis including the maturation of sperm cells and transport of glucose and protein synthesis into sperm cells resulting in a decrease in the integrity of sperm cell membranes [10]. Alkaloids can affect ATPase enzyme activity in spermatozoa cell membranes and further disrupt the homeostasis of sodium and potassium ions [20].

V. CONCLUSION

Carica papaya fractions (N-hexane, ethyl acetate and water) have antifertility effect on sperm motility. The decresed *Carica papaya* fractions (N-hexane, ethyl acetate and water) were 33 %, 65%, and 75% respectively. The water fraction has the greatest decrease in spermatozoa motility.

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