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Phytopharmaceutical modification of papaya (*Carica papaya*) seed extract on sperm quality using self-nano emulsion as contraception potential

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ABSTRACT

Carica papaya is known to reduce the quality of spermatozoa in morphology and viability of spermatozoa. Biochemical compound in Carica papaya is unstable and can easily degraded in disgetive track. Formulation of papaya seeds in the form of self-nano emulsion (SNE) is an alternative drug delivery system to increase bioavailability by lipophilic means. The sample consisted of 18 rats which were divided into 3 groups, namely the control group which was not given any treatment; the treatment group which was given papaya seed ethanol extract as much as 100 mg/kgBW and the treatment group which was given papaya seed extract-SNE as much as 100 mg/kgBW, the administration was carried out orally for 28 days. Examination of the quality of rat spermatozoa includes morphology, motility, and viability. Data analysis using One Way ANOVA and Chi-Square test. The results of the analysis showed that the decrease in morphology and viability of spermatozoa was significantly different with a result ($p < 0.05$) between treatment groups. As for motility, it does not have a significant difference with a result ($p > 0.05$) between treatment groups.. Based on the results of the study, it can be concluded that the administration of papaya seed ethanol extract and papaya seed SNE extract significantly reduces the quality of spermatozoa in the form of morphology and viability, and does not significantly reduce the motility of Wistar strain white rat spermatozoa.

INTRODUCTION

The population growth in Indonesia continues to increase every year, this is a major problem faced by developing countries. Indonesia is the fourth most populous country among the ten largest populations globally. The government seeks to reduce the birth rate by creating a family planning program named "KB". *Keluarga Berencana* (KB) is an effort to plan the number and spacing of pregnancies using contraception. The objective of KB is to establish

an appropriately modest family within the social economy by the planning of childbirth to achieve a successful household (Ruspawan et al., 2021).

Most contraceptive use is carried out by women, and the participation of men in the use of contraception is still low. One reason for the low participation of men in *KB* program is the limitation of contraception types that are available to men. There are also a very limited knowledge about reproduction health. Therefore, that is a necessary development of technology contraception especially for men (Ghaffarilaleh et al., 2019).

Efforts to find male contraception by utilizing natural have been carried out by experts but it's still far from the target. The results of the natural contraception cannot be used by the community at large, therefore the search and research related to contraception with natural material still need to be intensified. Medicinal ingredients for originating contraception from natural own profit among them toxicity low, easily obtained, affordable price and effect side little (Irawaty & Pratomo, 2019).

One material nature can used as contraception is papaya seeds (*Carica papaya*). Several studies prove that papaya seed affects antifertility study about giving methanol extract, n-hexane fraction, and seed methanol fraction papaya with a dose of 100 mg/ kg body weight influential to decline reproductive organ weight. More testicular weight is low because of disruption of growth and development cells spermatogenic in the reproductive organs of males (Nita et al., 2020).

Papaya seeds contain chemical compounds like group phenols, triterpenoids, flavonoids, alkaloids, and saponins. The chemical compound content of papaya seeds characteristic antifertility and can used as contraception in men (Nita et al., 2020). Saponins in seeds of papaya can role in the synthesis of steroid hormones and estrogen contraceptives. Flavonoids in the seeds of papaya can stimulate the formation of estrogen. The action of saponins and flavonoids of seeds papaya This gives bait come back negative to the hypothalamus-pituitary-testicles (Aritonang, 2019).

Giving papaya seed extract to Wistar rat spermatozoa decreases the quality of spermatozoa in the morphological aspect. On sperm found primary abnormality was due to impaired spermatogenesis when the formation of spermatozoa from spermatids. It is also found in sperm abnormality secondary allegedly disturbances in spermatozoa maturation in the epididymis (Nita et al., 2020).

Despite the beneficial effect of the papaya seed extract, the compound it self has a poor bioavaibility. This leads to the compound will be degraded when passing through the disgative tract. Formulation of papaya seeds in the Self Nano Emulsion (SNE) form is one alternative to increase bioavailability in a lipophilic form *Self-nano emulsion* is a formulation consisting of oil, surfactant, and co-surfactant in the right proportions to form a stable isotropic combination. SNE uses technology nanoparticles to increase absorption and availability inside the body, especially for compounds with limited water solubility (Erliyana et al., 2022).

METHODS

Tools and Instrumentation

Tools used in study this covers scales analytical (Ohaus®), oven (Memert UN110®), grinder (Maksindo®), rotary evaporator (IKA®), ultrasonic cleaner (GT Sonic R20®), tools glass (Pyrex® and Iwaki®), micropipettes (Dragon Onemed®), GC-MS (ISQ Series®), magnetic stirrer (C-Mag HS 7®), pH meter (Ohaus®), microscope (Olympus®), feeding tube, cage rat and data analysis software (SPSS®25.0).

Material

Materials used in study this covers papaya seeds (*Carica* papaya) rat white male strain Wistar, propylene glycol, tween 80, acid oleate, distilled water, 96% ethanol, and ether.

Extraction Papaya Seeds

Extraction of papaya seeds used ultrasonic-assisted extraction (UAE) methods. Extraction was done by mixing powder of papaya seeds with solvent 96% ethanol at 40 ℃ for 30 minutes with a frequency of 50 Hz. Then filter the solvent with paper filter Whatman No.42 for separate dregs papaya seeds. Filtrate concentrated with exhausted for evaporate solvent so that obtained extract thick papaya seeds (Laksono et al., 2022).

Characterization Extract with GC-MS

The column used is DB-5MS (non-polar column). Length 30 m, diameter 0.25 mm, temp injector 250℃, temp detector 280℃, program

scan 40°C/2 increments of 10°C per minute up to 280°C/3, pressure 68 Kpa, speed flow 0.9 mL/ minute, L. *velocity* 34.2. Samples will be tested filtered formerly. Then diluted 5 times to make as much as 1 mL of solution mixture using solvent ethanol. The sample is injected with 1 µL (El-Beltagi et al., 2019).

Preparation SNE Extract Papaya Seeds

SNE was created with dissolved extract papaya seeds with oil (oleic acid) use continued *vortex* with the sonication process for 15 minutes. Based on research by Nita *et al*. (2020), thick papaya seeds extract of 100 mg/KgBW weight is the optimum concentration for antifertility. Surfactant (Tween 80) was added then homogenized using *vortex* and sonication for 15 minutes. Co-surfactant (Propylene glycol) was added then homogenized using *vortex* and sonication for 15 minutes (Pratiwi et al., 2020). The formula for SNE is in **Table 1** the percentages in this table indicate the concentration of each 10 ml of the SNE.

Evaluation Preparation of SNE Extract Papaya Seeds

Measurement size particle done with tool *particle size analyzer* (PSA). Samples are included in the cuvette and read by the PSA (Shiyan et al., 2023; Pratiwi et al., 2024). Index polydispersity indicated uniformity in size globule preparation. The lower the polydispersity index value, the higher the uniformity of the globule size. Measurement was done using *particle size analyzer* (PSA). Samples are entered into in PSA cuvette, then monochromatic light is fired by the instrument, resulting in refracted light and output light scattering at an angle of 90^0 will captured by the detector so can produce diameter and index polydispersity (Wijaya et al., 2023).

Table 2. Group Test Treatment

Group	Treatment
Control	Rats do not give any treatment
K1	Rat given extract papaya seeds
K2	Rat given SNE extract papaya seeds

Pharmacologycal Assay

Administration of SNE extract papaya seeds and extract papaya seeds in rats orally using a 1 mL volume probe. Rats are still fed and watered during test SNE extract papaya seeds and extract papaya seeds given with calculated dose by mg/KgBW of rats. The treatment was done on 28th days and on the 29th day surgery was performed. The treatment was carried out in accordance with the test treatment groups in **Table 2**.

Table 1. Formula of SNE extract papaya seeds

Material	Formula
Oleic acid (mL)	1
Tween 80 (mL)	6
Propylene glycol (mL)	3
Papaya Seed Extract (mg)	0.05

Spermatozoa Preparations

Dissected rats were taken epididymis to examine the spermatozoa. Spermatozoa collection is carried out with clamp of the head and cauda of the epididymis then cutting the efferent duct, then sorting fluid sperm in petri dish that contained 2 mL of solution physiological solution (0.9% NaCl) at a temperature of 37-40°C and a spermatozoa suspension was obtained. Spermatozoa suspensions were taken with a drop pipette and dripped on the glass preparations. The dried preparations were then dripped with fixative solution in the form of methanol and left dry. The dried preparation was stained, first use eosin dye and then methylene blue. The final stage is washing with water (Irawan et al., 2023).

Counting Rat Spermatozoa Motility

Observation of motility from rat spermatozoa were made by immediately from the cauda epididymis. Spermatozoa were dripped on a glass object as much as one drop and try the same big for every inspection. The observation was done with microscope light with 400 times magnification. Moving spermatozoa observed and calculated how many times to move tail in one minute, rated based on the percent spermatozoa with motility good, namely moving spermatozoa straight forward, fast, agile, and active (Yucel etl.al., 2019).

Counting Viability of Rat Spermatozoa

Counting spermatozoa viability was carried out with dripped solution sperm as much

Table 3. Sperm abnormalities

Condition	Definition
Macro sperm	Head size larger that normal
Micro sprem	Head size smaller than normal
Double hand sperm	Two heads of vrying shapes and sizes
Amorphous sperm	Strange head shape the abnormal neck and center of sperm include as following
Sperm defect	The center of the sperm is twisted/broken
Loose head sperm	Headless sperm
Sperm coil	Twisted tail section
Double tail sperm	Double tail

as one drop on the glass object. The sample later dripped one drop of eosin-Y solution and mixed then closed. The observation was done with microscope light 400 timesmagnification. Percentage number of live spermatozoa of 100 spermatozoa for every repetition. Spermatozoa die be marked with the color red (Jalili et al., 2018).

Counting Morphology of Rat Spermatozoa

According to Yang etl.al., (2024) morphology of normal sperm has length and width with regular limits. The head part is related to the shaped month scythe and the tail is long no roll-up (**Table 3**). Observation morphology sperm done with preparation deletions made with dripping sperm on a glass object, preparations are made, removed, and dried. Preparation deletion was fixed with methanol for 3-5 minutes and colored with *Giemsa* 3% for 30 minutes. The preparation is washed with distilled water and dried at temperature room. The observation was done with microscope light 400 times magnification of 200 sperm and the results stated in percent with formula percentage as follows:

RESULT AND DISCUSSION

Papaya Seeds Extracts Characterization

The papaya seeds extract obtained in this study is golden brown in color, oil-like and thick in texture (**Figure 1a**). Gas chromatography has wide applications and can be made as a separation and analysis mixture of several components. Gas chromatography can read compounds with lowest concentration so that metabolites secondary in plants can be identified with results from chromatograms and spectrum mass. The gas chromatography results show a chromatogram from extracted ethanol papaya seeds (**Figure 2**).

The most compound component in extract papaya seeds is located at *peak* 6 with a retention area amounting to 43.09%. Possibility three compounds are cis-13- Octadecenoic acid, cis-vaccenic acid, and trans-13-Octadecenoic acid which include in fatty acids. Papaya seed (20.97-30.10%) are enriched with lipophilic phytochemicals and essential fatty acids like sour oleate (70.84%-79.10%) (Dotto & Abihudi, 2021). Based on the phytochemical screening test on papaya seed ethanol extract, it shows that flavonoid saponins are contained which can play a role in reducing the quality of spermatozoa.The most compound component in extract papaya seeds is located at *peak* 6 with a retention area amounting to 43.09%. Possibility three compounds are cis-13- Octadecenoic acid, cis-vaccenic acid, and trans-13-Octadecenoic acid which include in fatty acids. Papaya seed (20.97-30.10%) are enriched with lipophilic phytochemicals and essential fatty acids like sour oleate (70.84%-79.10%) (Dotto & Abihudi,

Figure 1. Development flow of SNE papaya seed extract based contraception

2021). Based on the phytochemical screening test on papaya seed ethanol extract, it shows that flavonoid saponins are contained which can play a role in reducing the quality of spermatozoa.

Preparation of SNE Extract Papaya Seeds

SNE shows a huge potential to overcome associated limitations with multiple oral administration compounds. Limitations among them low solubility in the channel digestion,

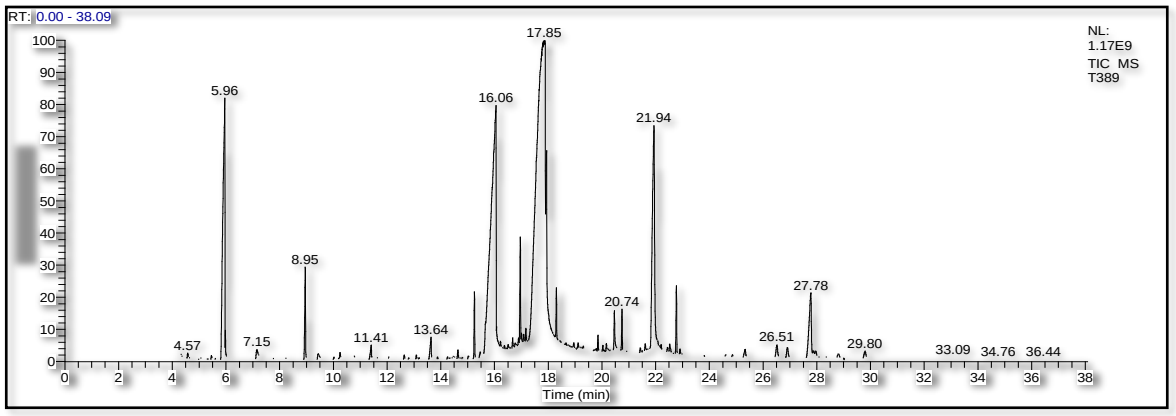

Figure 2. Chromatogram extract ethanol papaya seeds. Analysis results show that there are 12 *peaks* and 36 possibilities component successful compound extracted from solvent ethanol with Different *Similarity Index (SI)* (Table 4).

Table 4. Indentified Compounds Using GC-MS from Papaya Seeds Extract

Peak	Real-Time	Hit 1	Hit 2	Hit 3	Retention Area (%)
1	5.95	Benzene, 1-isocyanic-2-methyl- SI: 929	Benzyl nitrile SI: 924	2,4,6-Cycloheptatriene-1-carbonitrile SI: 908	11.39
2	8.95	Benzene, (isothiocyanatomethyl)- SI: 906	Thiocyanic acid, phenylmethyl ester SI: 859	Benzyl 2-chloroethyl sulfone SI: 787	1.60
3	15.25	Hexadecanoic acid, methyl ester SI: 924	Pentadecanoic acid, 14-methyl-, methyl ester SI: 865	Pentadecanoic acid, methyl ester SI: 830	0.92
4	16.04	n- Hexadecanoic acid SI: 864	l-(+)-Ascorbic acid 2,6-dihexadecanoate SI: 859	Pentadecanoic acid SI: 815	21.28
5	16.96	trans-13-Octadecenoic acid, methyl ester SI: 922	9-Octadecenoic acid (Z)-, methyl ester SI: 920	cis-13-Octadecenoic acid, methyl ester SI: 918	1.83
6	17.85	cis-13-Octadecenoic acid SI: 939	cis-Vaccenic acid SI: 935	trans-13-Octadecenoic acid SI: 918	43.09
7	17.93	Octadecanoic acid SI: 837	Octadecanoic acid, 2-(2-hydroxyethyl)ethyl ester SI : 772	6-Octadecenoic acid, (Z)- SI: 767	2.66
8	20.46	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester SI: 899	Glycerol 1-palmitate SI: 868	Octadecanoic acid, 2,3-hydroxypropyl ester SI: 788	1.04
9	20.74	Diisooctyl phthalate SI: 854	Phthalic acid, di (2-propyl pentyl) ester SI: 849	Bis(2-ethylhexyl) phthalate SI: 830	0.69
10	21.93	2,3-Dihydroxypropyl elaidate SI: 879	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester SI: 859	Oleic acid, 3-hydroxypropyl ester SI : 858	11.34
11	22.77	Squalene SI: 928	2,6,10,14,18-Pentamethyl-2,6,10,14,1 8-eicosapentaene SI: 848	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)- SI:844	0.99
12	27.77	ç-Sitosterol SI: 927	á-Sitosterol SI: 816	5-Cholestene-3-ol, 24-methyl- SI: 763	3.17

solvents are not consistent, degradation enzymatic, and intestinal absorption is not uncertain. Surfactants and lipid components used in SNE can work the same for increased absorption of gastrointestinal drugs. Apart from that, the components can modified by the need to make a decent SNE for hydrophilic and hydrophobic (Buya et al., 2020). SNE

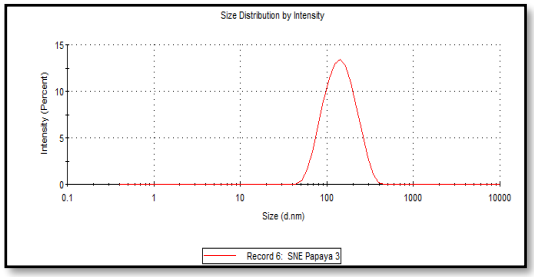

Figure 3. Distribution size of droplet from nano emulsion

formulation is capable of increasing rate dissolution from substance given active orally is one of the consequences of increased interface area oil and water so release solubility and bioavailability (Buya et al., 2020). SNE papaya seeds extract in this study used oleic acid as oil, tween 80 as a surfactant, and propylene glycol as a co-surfactant. The success of the SNE formula can be seen from the visual clarity and no phase separation which can be seen in **Figure 1c.**

Evaluation Preparation of SNE Extract Papaya Seeds

The size of nanoparticles in the self-nano emulsion preparation of papaya seeds extract is 116.9 nm **(Figure 3)**. this fulfills the requirements of nanoparticles in the range of 10-1000 nm. The optimum ultrasonication time is judged by the ability to dissolve and distribute an ingredient evenly in the system. The longer mixing time, the smaller particle size. This is due to the longer duration of the ultrasonic radiation forces to disperse the colloidal droplets to smaller sizes (Abdallah et al., 2020

Polydispersity index value indicates the uniformity of the particles size. The mark index polydispersity so the size particle the more homogeneous. The polydispersity index value shows the size distribution that can be affect drug delivery, drug release and nanoparticle stability. Index polydispersity close to 0 indicates size more and more particles are uniform and homogeneous. Index polydispersity This gives information about stability physique something system dispersion formed nature more stable in the long term (Sapiun et al., 2023). Index polydispersity from SNE extract preparation papaya seeds is 0.226. Test results particles size and polydispersity index show that this SNE preparation was good **(Figure 3)**.

Pharmacologycal Assay

Extract was carried out using test animals in the form of rats divided into three treatment groups. The first group was used as a comparison, namely rats that were not given any treatment. The second group was used as a test of papaya seed extract (suspended with Na CMC) as much as 100 mg / 200 g. The third group was used as a test of papaya seed SNE extract 100 mg / 200 g. In this study there was no test group with empty SNE because the three test groups were sufficient to see the effect of papaya seed extract on reducing the quality of rat spermatozoa, maybe for further research the test group could be added. The quality of spermatozoa after testing is seen from the aspects of morphology, motility, and viability.

Spermatozoa Morphology

Morphological parameters of spermatozoa show a daflation in the group extracted papaya seed and SNE extract papaya seed compared to the normal group. As what we can see in **Table 5** This thing caused by extracts of papaya seed cause abnormalities in parts of spermatozoa neck and impacts on function producing mitochondria energy no maximum (Aritonang, 2019). Based on value test results normality and homogeneity in a way, statistics show that each data is distributed normally and homogeneously. This thing shows that data fulfill the condition for the One-way ANOVA test. The results of the parametric test analysis (One Way ANOVA) show a decline in spermatozoa morphology that occurs differently significantly with results ($p < 0.05$) between group treatments.

Spermatozoa Motility

Table 5 Morphology, motility and viability values of rat spermatozoa from each treatment

Group	Morphology (%)	Motility (%)	Viability (%)
Normal	37.87 ± 0.87	64.07 ± 1.43	77.71 ± 1.12
K1	33.71 ± 0.79	59.68 ± 0.66	65.48 ± 1.21
K2	35.34 ± 1.84	62.87 ± 1.41	69.01 ± 1.47

Sperm motility parameters show decline in the group extracted papaya seed and SNE extract papaya seed compared to the normal group. The motility of sperm is a parameter of the continuity of life of sperm. This thing still there is a connection with spermatozoa morphology that mitochondria produce minimum energy and affect spermatozoa motility. Spermatozoa are considered normal if their motility is more than 50% and spermatozoa are said to be abnormal if amount percentage the abnormality is not enough from 40% (Dias et al., 2019). Measurement results in spermatozoa motility in research This No there is something below 50% which indicates that spermatozoa are included in the normal category, however still there is still a decline in spermatozoa motility. Decreased spermatozoa motility can happen because function mitochondria produce energy no maximum. That thing happens because of abnormality of organelle cells in parts of the neck of spermatozoa, is abnormality structure from neck bending and vacuolization of mitochondria. This spermatozoa abnormality happens because exists impaired spermatogenesis in the spermiogenesis phase (Busman et al., 2020). Based on *Chi-Square* test results in a way, statistics show that each data does not distribute normally and homogeneously. The results of the non-parametric test analysis (*Chi-Square Test*) show a decline in spermatozoa motility that occurs with no different significance with the result of asymptotic significance (2-sided) 0.375 > 0.05, where condition asymptotic significance value (2-sided) < 0.05.

Spermatozoa Viability

Viability parameters also show the same result as parameters morphology and motility parameter happen to decrease in treatment extract papaya seed and SNE extract papaya seeds. Viability shows the ability of spermatozoa to survive life after being issued from the reproductive organs. This thing because the papain enzyme is contained in papaya seed. Papain can damage organelle cells Sertoli and spermatogenic cells in the process of spermatogenesis (spermatogonia, spermatocytes, spermatids, and spermatozoa) (Syamsuddin et al., 2021). Based on value test results normality and homogeneity in a way, statistics show that each data was distributed normally and homogeneously. This thing shows that data fulfill the condition for the One-way ANOVA test. The results of the parametric test analysis (One Way ANOVA) show decline in spermatozoa viability occurs differently with results ($p < 0.05$) between group treatments.

Based on results, extract of ethanol papaya seeds and SNE extract papaya seeds can used as a contraception experience for men. Extract papaya seeds has a better effect as a contraceptive and then papaya compared to SNE extract papaya seeds. Extract papaya seeds influences morphology and viability of spermatozoa from rats. Seed-content papaya is believed can cause a decline in morphology, motility, and viability in rat spermatozoa including alkaloids, papain enzymes, saponin compounds, flavonoids, steroids, and triterpenoids. Saponins in seeds of papaya can role in the synthesis of steroid hormones and estrogen contraceptives. Flavonoids in the seeds of papaya can stimulate the formation of estrogen. The action of saponins and flavonoids on seeds papaya This gives bait come back negative to the hypothalamus-pituitary -testes. Gonadotropin activity with mechanism bait comes back negative from the hypothalamus male nor female can lower secretion of *Gonadotropin Releasing Hormone* (GnRH). Decreased GnRH secretion causes secretion of *Follicle Stimulating Hormone* (FSH) and *Luteinizing Hormone* (LH) is also involved in decreased. Decreased FSH and LH secretion will result in spermatogenesis disorders. Papain content in seeds of papaya can decrease total lipids in testicular and epididymal tissue and decrease the activity of lipoprotein lipase enzyme. The declining activity of the lipoprotein lipase enzyme will have an impact on the process of spermatogenesis in the testes and the maturation of spermatozoa in the epididymis (Aritonang, 2019).

CONCLUSIONS

SNE-papaya seed extract formulation has the ability to reduce the number of spermatozoa in rats, which indicates a negative effect on sperm production and has the potential as a contraceptive. In addition, administration of SNE papaya seed extract affects spermatozoa motility and viability, so it can reduce the ability of sperm movement to reach the egg and reduce sperm survival which can affect fertility. Modification of papaya seed extract in the form of SNE has the

potential to reduce the quality of rat spermatozoa better.

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CONFLICT OF INTERESTS

No conflict of interest

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