Poll Res. 40 (1) : 335-340 (2021)

ISSN 0257–8050

EFFECT OF GUAVA FRUIT (*PSIDIUM GUAJAVA* L.) EXTRACT TO THE MOTILITY AND VIABILITY SPERMATOZOA OF RATS (*RATTUS NOVERGICUS*) EXPOSED TO CIGARETTE SMOKE

EARLY JUVITTA ANGGRAINI, WURLINA* AND JOLA RAHMAHANI

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

(Received 18 May, 2020; Accepted 26 July, 2020)

ABSTRACT

The purpose of this study was to determined the effect of giving guava fruit extract (*Psidium guajava* L.) to the motility and viability of spermatozoa of albino rats (*Rattus norvegicus*) exposed to cigarette smoke. The type of this study was an experimental study with a complete random design. This study used 25 albino rats with 2-3 months age group, male and weighing 200 gram of body weight (BW). The negative control group (C-) was given CMC Na 0.5% 2 mL/head without exposure to cigarette smoke. P0 group was given exposure to cigarette smoke and CMC Na 0.5% 2mL/head. The treatment groups P1, P2 and P3 were given guava fruit extract (*Psidium guajava L.*) at a dose of 18.9 mg/kg BW; 37.8 mg/kg BW; 56.7 mg/kg BW and exposed to cigarette smoke. The results of spermatozoa quality research using the ANOVA test showed a significant difference ($p< 0.05$) between the control group and the treatment groups. The results showed that guava fruit extract (*Psidium guajava* L.) improved the motility and viability of spermatozoa exposed to cigarette smoke. It was concluded that the dose of 56.7 mg/kg BW gives the best effect to improved and increased the motility and viability of spermatozoa. Suggestions submitted by research to find out more about the effect of giving exposure to cigarette smoke on spermatozoa quality, it is necessary to test HOS (hypoosmotic swelling) to determine the integrity of the spermatozoa plasma membrane.

KEY WORDS : Guava fruit extract, Spermatozoa, *Rattus novergicus,* Cigarette smoke

INTRODUCTION

Cigarettes are addictive substances that are harmful to human health. The main ingredient in cigarettes is tobacco. Tobacco contains approximately 4000 elements and at least 200 of them dangerous health. The main poisons in tobacco and can provide effects that interfere with health, among others nicotine, tar, carbon monoxide gas and various heavy metals (Fitri, 2013). According to the world health organization World Health Organization (WHO), by 2020 it is estimated that 10 million smokers in the world will die each year (Putra, 2016). Data from the Global Adult Tobacco Survey (GATS) states that the prevalence of active smokers in Indonesia is 67% in men and 2.7% in women, while the prevalence of passive smoking, which is 40.5% with more than half are women and toddlers (Prameswari, 2014).

Cigarettes are one of the causes of damage to cells because cigarettes contain substances that can form free radicals. Free radicals are products that are formed in various chemical reaction processes in the body (cell metabolism, breathing, excessive exercise) and can also be formed from environments polluted by cigarette smoke, vehicle fumes, pollutants and also radiation (Putri, 2015)

A study conducted on albino rat (*Rattus norvegicus*) exposed to 4 cigarettes/day for 36 days, causing a decrease in the quality of spermatozoa (Putra, 2016). Decreased quality of spermatozoa can be caused by the presence of oxidative stress conditions caused by excessive amounts of free radicals (Muliartha, 2009). Free radicals damage cells by damaging cell membranes with increase the amount of lipid peroxidation (OH*) and cause damage and decrease the integrity of the

spermatozoa membrane (Sari, 2014). Spermatozoa plasma membrane composed of unsaturated fatty acids susceptible to oxidative damage due to free radicals, damage to the cell plasma membrane negatively impacts the viability and motility of spermatozoa (Durairajanayagam, 2014).

Living bodies need antioxidants as protection to against free radicals. Lack of antioxidants in the body can cause free radicals, in this case the antioxidants more easily carry out oxidative damage (Durairajanayagam, 2014). Antioxidants are divided into two, namely synthetic antioxidants (derived from chemical reactions) and natural antioxidants that are easily obtained from a variety of herbal ingredients (fruits and plants). One ingredient that is useful to ward off free radicals and improve antioxidants in the body is guava fruit (Muslihah, 2000).

Guava (*Psidium guajava* L.) is a fruit that can be used as functional food because it has a good function for health. Guava fruit has been used emipiris for traditional medicine and many herbal products are made from guava fruit preparations. Guava which has the best antioxidant potential is white fleshy guava which is extracted with 70% ethanol by maceration (Purwandari, 2018). The functional properties of guava are caused by the presence of a high vitamin C content (Paniandy, 2000). Vitamin C is a non-enzymatic antioxidant that has high polarity properties, because it contains a lot of hydroxyl groups so that it dissolves easily in water. Therefore, this vitamin is in extra cellular fluid. This provides an advantage because it is easily changed by body. Therefore, vitamin C can react and be able to neutralize free radicals (Wibisono, 2001).

The active ingredient contained in guava fruit is expected to reduce the production of free radicals and increase the motility and viability of albino rat spermatozoa (*Rattus norvegicus*) which are decreased due to exposure to cigarette smoke. Based on the above background, the researchers wanted to test the potential of guava fruit extract (*Psidium guajava* L.) for increased motility and viability of spermatozoa of albino rat (*Rattus norvegicus*) exposed to cigarette smoke.

MATERIALS AND METHODS

This study used 25 male albino rats (*Rattus norvegicus*) animals, aged 2-3 months with a body weight of 200 grams, red salt cellar clove cigarettes. Material used to make guava fruit extract (*Psidium guajava* L.): guava fruit powder, sterile aquadest, ethanol 96%, CMC Na 0.5%, chicken feed in the form of pellets for rat feed, drinking water, sawdust for rat cage pads. The tools used in this study include a smoking box that has two connecting holes in the front, which are connected by hose and syringe.

25 male rats were divided into 5 groups so that each group consisted of 5 rats was adapted for 7 days so that the experimental animals did not experience stress. In the negative control group (C-) no smoke exposure was given and only CMC Na 0.5% 2ml/head. On the 8th day the treatment began with 3 cigarettes per day in cigarette P0, P1, P2 and P3 groups; then the P0 group was only given CMC Na 0.5%, guava fruit extract was given with 3 different doses for P1, P2 and P3 namely amounting to a dose of 18.9 mg/kg body weight, 37.8 mg/kg BW, 56.7 mg/kg BW per day. Exposure to cigarette smoke is done by inserting each group of rats in the smoking box connected by a hose, cigarettes are burned and smoked using a 60cc syringe, a syringe containing cigarette smoke is injected into the box, a total of 1 cigarette is burned as much as 32 suction and 1 cigarette spend about 5 minutes. Exposure to cigarette smoke and administration of guava fruit extracts were carried out for 45 days and on the 46th day all mice were sacrificed and their epididymal organs were taken and motility and viability were examined using a 400x magnification microscope.

Check the percentage of spermatozoa cell motility by dripping one drop of spermatozoa suspension onto a glass object and examined under a microscope at 400x magnification. The results of the study are based on three fields view and observe the progressive movements of individual spermatozoa that are seen and a percentage of spermatozoa that moves to the total number of spermatozoa seen in each field of view.

Inspection of spermatozoa cell viability percentage by dripping spermatozoa suspension on glass objects and one drop of negosin Eosine solution in addition to droplets of spermatozoa cell suspension, then mixed until homogeneous using sterile ose. The result of a mixture of suspension and eosin is pushed to form a thin coverage to the other end, then the preparation is dried over a flame. The preparations are observed under a 400x magnification microscope. The results of the study are based on three fields of view and observed live spermatozoa against the total number of spermatozoa seen in each field of view. Living

spermatozoa will not be stained by dyes, while dead spermatozoa will be colored. Dead spermatozoa will look purplish red, while living spermatozoa will appear white without color.

The data obtained will be arranged in a table which then the motility and viability of spermatozoa cells will be analyzed by ANOVA and followed by Duncan Multiple Range Test (DMRT) to determine the best treatment.

RESULTS

Spermatozoa Motility

The average result of the highest motility percentage is $77.57^{c} \pm 11.37$ owned by the negative control group and shows the real difference between each training group. In the P0 treatment showed a decreased percentage of spermatozoa motility with a negative control group that is $37.38^{a} \pm 6.73$. Furthermore, in P1, P2 and P3 showed an increase in all groups, namely P1 = $57.13^{b} \pm 17.73$, P2 = $58.84^{b} \pm 15.07$, and P3 = $62.44^{bc} \pm 11.69$ which can be seen in Table 1.

The results showed that there were significant differences in each group C(-), P0, P1, P2 and P3. P0 group exposed using cigarette smoke and without guava fruit extract had lower percentage results than group C(-). This proves that cigarette smoke that is inhaled every day can affect the decrease in spermatozoa motility (Agarwal, 2003).

In group P1, there was an increase in spermatozoa motility percentage compared to group P0, this proves that the administration of guava fruit extract as a therapy at the lowest dose in this study was able to have a positive effect in increasing the percentage of spermatozoa motility, but the percentage that appeared to be insufficient to restore the percentage of spermatozoa motility which decreased to normal again. Compared to P1, there was no significant increase in percentage results, but at this dose it was able to increase the percentage of spermatozoa motility which decreased due to exposure to cigarette smoke.

Table 1. The mean percentage of spermatozoa motility of albino rats (*Rattus norvegicus*) exposed to cigarette smoke.

Treatment Groups	Mean ± SD
C-	$77.57^{c} \pm 11.37$
P0	$37.38^{a} \pm 6.73$
P1	$57.13^{b} \pm 17.73$
P2	$58.84^{b} \pm 15.07$
P3	$62.44^{bc} \pm 11.69$

Note: Different superscripts in the same column show significant differences ($p < 0.05$).

In the P3 group which was the group with the highest dose in this study, there was a significant increase compared to the other treatment groups. The results of the spermatozoa motility percentage showed that giving guava fruit extract with high doses can increase the percentage of spermatozoa motility and can restore the percentage of spermatozoa motility decreases to normal again. This is due to the content contained in guava, can react positively in warding off free radicals. Antioxidants contained in guava fruit extract can stabilize free radicals by completing the lack of electrons possessed by free radicals and inhibit the chain reaction of the formation of free radicals that can cause cell damage. Thus damage to cell membranes due to free radicals can be inhibited, so that the process of spermatogenesis is more optimal and an increase in spermatogenic cells (Arsana, 2014).

Quercetin compounds and vitamin C has an effect against ROS caused by cigarette smoke which can deactivate Superoxyde Dismutase (SOD) and Catalase (CAT), and can cause and influence the activities of SOD and CAT. The decline SOD activity is due to its reaction with O2 to form H_2O_2 and O_2. The effects of quercetin and vitamin C can reduce excessive ROS production (Putri, 2015). The combination of quercetin and vitamin C compounds can protect the phospholipid layer with non-covalent bonds, this way can increase the regeneration of endogenous antioxidants (Paunoviæ, 2016).

Flavonoids have ability as an antioxidant can inhibit oxidative stress against the dangers of free radicals and can enhance the process of spermatogenesis. The effect of flavonoids can also increase regeneration by deterring free radicals, providing competitive substrates for unsaturated lipids in membranes and or accelerating the repair mechanism of damaged cell membranes (Sarma, 2011).

Spermatozoa Viability

According to the Table 2, the mean percentage of the highest viability results is $90.05^{c} \pm 3.015$ which is owned by the negative control group and showed significant differences in each treatment group. In

treatment P0 showed a decreased percentage of viability spermatozoa with a negative control group that is $40.23^{a} \pm 13.71$. Furthermore, the treatment P1, P2 and P3 showed an increase in all treatment groups, which are P1 = $56.82^{b} \pm 10.31$, P2 = $62.44^{b} \pm 18.75$, and P3 = $68.07^{b} \pm 6.93$.

The results of the spermatozoa viability study showed that there were significant differences in groups C-, P0, P1, P2 and P3. P0 group exposed using cigarette smoke and without guava fruit extract had lower presentation results than group C.

Exposure to cigarette smoke can reduce spermatozoa concentration and sperm viability and increase spermatozoa abnormalities (Unitly, 2014). Decreased presentation of spermatozoa viability due to damage to the spermatozoa plasma membrane. When spermatozoa are exposed to a hypoosmotic condition then the water will enter the spermatozoa to reach the osmotic state, as a result the volume of spermatozoa increases and the plasma membrane is damaged. Damage to vital substances in spermatozoa causes intracellular enzymes, lipoproteins, ATP, intracellular potassium and fat is reduced so that membrane permeability is disrupted (Kosasih, 2019). As is well known membrane permeability iṡ closely related to the transportation of nutrients whose important role in cell metabolism. Disruption of the permeability of the spermatozoa membrane then nutritional needs will be disrupted and subsequently result in dead spermatozoa (Sopiyana, 2006).

In group P1, there was an increase in spermatozoa viability compared to group P0, this proves that administration of guava fruit extract as a therapy at the lowest dose in this study was able to have a positive effect in increasing percentage viability of spermatozoa, but the percentage that appears to be insufficient to restore the percentage of spermatozoa viability decreased to normal again, Likewise with the P2 group which is a group with dosing increased twice compared to P1, there was no significant increase in the percentage results, but in the dose this can increase the percentage of spermatozoa viability which decreases due to exposure to cigarette smoke.

In group P3 which is the highest dose in this study, a significant increase compared to other treatment groups. The spermatozoa viability percentage results showed that the administration of high doses of guava fruit can increase the percentage of spermatozoa viability better among other treatment groups, but at the highest dose this is still not able to restore percentage Viability is back to normal again. This can occur if the dose given has not been able to restore the viability of spermatozoa to normal again, but to start neutralizing free radicals in the body can already occur by observing the increase in viability of spermatozoa in groups P1, P2 and P3.

This proves that guava fruit extract can improve the viability of albino rats (*Rattus norvegicus*) which decreases due to oxidative stress. Guava fruit contains vitamin C, vitamin B1, vitamin A, protein, fat, carbohydrates, water, calcium and phosphorus. In guava fruit also found polyphenols, namely tannins, manganese, saponins, flavonoids, guajavarin, and quercetin. Flavonoids have the ability as antioxidants that can inhibit oxidative stress fight the dangers of free radicals and can enhance the process of spermatogenesis (Bakti, 2010; Sarma, 2011).

The effect of flavonoids can also be enhance regeneration by detoxifying free radicals, providing

Table 2. The mean percentage os spermatozoa motility of albino rats (*Rattus norvegicus*) exposed to cigarette smoke.

Treatment Groups	Mean ± SD (mg/dl)
C-	$90.05^{c} \pm 3.015$
P0	$40.23^{a} \pm 13.71$
P1	$56.82^{b} \pm 10.31$
P2	$62.44^{b} \pm 18.75$
P3	$68.07^{b} \pm 6.93$

Note: Different superscripts in the same column show significant differences ($p < 0.05$).

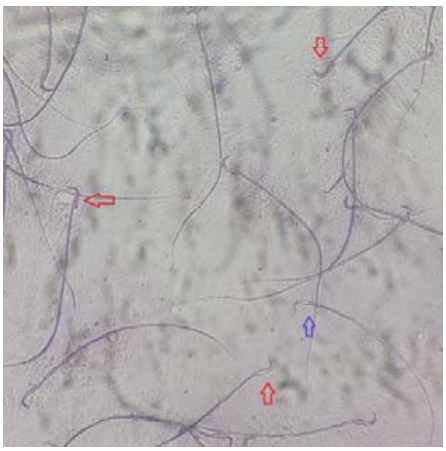

Fig. 1. Viability results of albino rat spermatozoa (*Rattus norvegicus*) using a 400x magnification. Blue arrow shows live Spermatozoa (Transparant) and red arrow shows died Spermatozoa (Colored *eosine nigrosine*).

competitive substrates for unsaturated lipids in membranes and accelerating the repair mechanism of damaged cell membranes. Quercetin compounds can inhibit oxidative stress by regulating the balance between oxidants and antioxidants. Quercetin effectively protects cells from free radical damage by increasing endogenous antioxidant levels. With the presence of antioxidants in guava fruit extract can be used as a free radical scavenger. If excessive free radicals in the body can already be captured by antioxidants, then cells that have been damaged by free radicals can regenerate themselves (Dewi, 2019).

The results of spermatozoa viability examination of albino rats (*Rattus norvegicus*) examined in a 400x magnification microscope can be seen in Figure 1.

CONCLUSION

The conclusion that can be drawn from this study is the provision of guava fruit extract can increase the motility and viability of white rat spermatozoa which are decreased due to exposure to cigarette smoke. The most influential dose was in the P3 group which was 56.7 mg/kg BW. The active content contained in guava fruit extract (*Psidium guajava* L.) can significantly increase the motility and viability of white spermatozoa which are decreased due to exposure to cigarette smoke by reducing lipid peroxidation and restoring antioxidant function in the testes.

ACKNOWLEDGEMENT

This study would like to give high gratitute to the all institutions that are involved which are Faculty of Veterinary Medicine, Universitas Airlangga for giving permission and support during this research process.

REFERENCES

Agarwal, A., Saleh, R.A. and Bedaiwy, M.A. 2003. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility*. 79 (4) : 829-843.

Arsana, I.N. 2014. Mangosteen (*Garcinia mangostana L.*) peel extract and physical training reduce oxidative stress in Wistar rats (*Rattus norvegicus*) during maximum physical activity. *Disertasi, Universitas Udayana, Denpasar*. [Text in Indonesian].

Bakti, K.K. 2010. *Protective Effects of White Guava (Psidium guajava L.) juice on Histological Damage of Gastric Mucosa Induced by Aspirin.* (Undergraduate Thesis, Universitas Sebelas Maret Surakarta). [Text in Indonesian].

DEWI, D.D. 2019. *The effect of administering guava extract (psidium guajava l.) on the diameter and thickness of epithelial tubules seminiferus testicles of albino rats (rattus norvegicus) with cigarette smoke.* (Undergraduate Thesis, Universitas Airlangga). [Text in Indonesian]

Durairajanayagam, D., Agarwal, A., Ong, C. and Prashast, P. 2014. Lycopene and male infertility. *Asian Journal of Andrology.* 16 (3) : 420.

Fitria, F., Triandhini, R.R., Mangimbulude, J.C. and Karwur, F.F. 2013. Smoking and DNA Oxidation. *Sains Medika: Jurnal Kedokteran dan Kesehatan*, *5*(2), pp.113-120. [Text in Indonesian].

Kosasih, K.A., 2019. *Antioxidant potential of red dragon fruit extract (hylocereus polyrhizus) to the number of spermatogenic cells and the sertoli cells of male mice (mus musculus) with exposure to high temperature.* (Undergraduate Thesis, Universitas Airlangga). [Text in Indonesian].

Muliartha, I.K.G., Sriwahyuni, E. and Yuliawati, Y. 2009. Administration of a combination of vitamin C and E orally improves liver damage due to exposure to sub-chronic clove cigarette smoke. *Jurnal Kedokteran Brawijaya*. 25 (1) : 23-27. [Text in Indonesian].

Muslihah, H. 2000. Natural antioxidants and free radicals: potential and application in health. *Kanisius. Yogyakarta*. 281. [Text in Indonesian].

Paniandy, J.C., Chane-Ming, J. and Pieribattesti, J.C. 2000. Chemical composition of the essential oil and headspace solid-phase micro extraction of the guava fruit (*Psidium guajava* L.). *Journal of Essential Oil Research*. 12 (2) : 153-158.

Paunoviæ, M.G., Ognjanoviæ, B.I., Matiæ, M.M., Štajn, A.Š. and Saièiæ, Z.S. 2016. Protective effects of quercetin and vitamin C against nicotine-induced toxicity in the blood of Wistar rats. *Archives of Industrial Hygiene and Toxicology*. 67 (4) : 304-310.

Prameswari, Y.N., Witjahjo, B. and Wijayahadi, N. 2014. The effect of multivelev dosing of honey towards the microscopic images of the lung on male mice Balb/C strains exposed to cigarette smoke. *Jurnal Kedokteran Diponegoro*. 3(1). [Text in Indonesian].

Purwandari, R., Subagiyo, S. and Wibowo, T. 2018. Antioxidant activity test of guava leaf extract (*Psidium guajava* L.). *Walisongo Journal of Chemistry*. 2 (2). [Text in Indonesian].

Putra, Y. 2016. The influence of cigarettes towards the number of spermatozoa mice (*Mus musculus*) Japanese strains. *Sainstek: Jurnal Sains dan Teknologi*. 6 (1) : 30-42. [Text in Indonesian].

Putri, A.P. 2015. The effect of vitamin C on the quality of spermatozoa exposed to cigarette smoke. *Jurnal Majority*. 4 (1). [Text in Indonesian].

Putri, A.P. 2015. The effect of vitamin C on the quality of

spermatozoa exposed to cigarette smoke. *Jurnal Majority*. 4 (1). [Text in Indonesian].

Sari, P.D., 2014. Effect of cigarette smoke in quality and quantity spermatozoa. *Jurnal Majority*. 3 (7).

Sarma, D.S.K. and Babu, A.V.S. 2011. Pharmacognostic and phytochemical studies of *Ocimum americanum*. *J. Chem. Pharm. Res*. 3 (3) : 337-347.

Sopiyana, S., Iskandar, S., Susanti, T. and Yogaswara, D. 2006. September. Effect of DMA, DMF and Glycerol cryoprotectants on the freezing process of Kampung Chicken Cement. In: *Seminar Nasional Teknologi Peternakan dan Veteriner*. [Text in Indonesian].

Unitly, A.J.A., Kusumorini, N., Agungpriyono, S., Satyaningtijas, A.S. and Boediono, A. 2014. Changes in the quality of spermatozoa and the number of spermatogenic cells of rats exposed to cigarette smoke. *Jurnal Kedokteran Hewan-Indonesian Journal of Veterinary Sciences*. 8 (2). [Text in Indonesian].

Wibisono, M. 2001. Effect of vitamin C on spermatid counts in *Mus musculus* exposed to ultrasonic waves. *Jurnal kedokteran YARSI.* 9 (1) : 96-103. [Text in Indonesian].