Effect of salam (Syzygium polyanthum Wight. Walp.) leaf extract on spermatozoa motility in ethanol-induced mice

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Abstract. Decreased quality of spermatozoa can occur because of the formation of free radicals resulting from ethanol induction. Salam [Syzygium polyanthum (Wight.) Walp.] leaves have been reported to have antioxidant activity. However, it is not known whether this antioxidant activity can overcome the decline in spermatozoa motility. Therefore, a study was conducted to investigate the effects of ethanol extracts obtained from salam leaves on spermatozoa motility in ethanol-induced mice (Mus musculus L.). Animals were divided into 5 groups. The normal control group (KK1) was not treated and the treatment control group (KK2) was administered with 0.5 % carboxy methyl cellulose (CMC) and ethanol (40 % v/v) at 2.8 g/kg body weight (bw). Ethanolic extracts of salam leaves at doses of 43.75, 87.5 and 175 mg/kg bw were orally administered into the mice in treated group 1 (KP1), treated group 2 (KP2), and treated group 3 (KP3), respectively. All treated mice were induced daily by ethanol per oral (p.o) at a dose of 2.8 g/kg bw (40 % v/v). The treatment was given for 20 consecutive days. The results of LSD testing showed that ethanol extracts of salam leaves resulted in significantly (P < 0.05) higher percentages of spermatozoa motility. Compared to the mice of the KK2 group (44.88 % ± 3.40 %), the spermatozoa motility of all treated groups (KP1 56.97 % ± 2.66 %; KP2 58.19 % ± 5.33 %; KP3 53.77 % ± 4.00 %) showed higher percentages of motility.

Keywords: Salam leaves, antioxidant, mice, spermatozoa, motility

1. Introduction
Unhealthy lifestyle choices, such as consuming alcoholic drinks and smoking, might increase free radical formation inside the body [1]. The imbalance of free radical generation and antioxidant defenses inside the body can cause oxidative damage to biomacromolecules, such as lipids, proteins, and DNA [2]. Such damage occurs when free radical molecules, which have high reactivity to gain electrons, attack stable molecules such as biomacromolecules. These stable molecules then become radical molecules, resulting in a radical chain reaction [3].

Ethanol is known to cause severe damage inside the body, such as lowering blood plasma testosterone and dihydrotestosterone (DHT) in epididymis, thus consequently lowering the motility percentage of mobile spermatozoa [4]. Ethanol may adversely affect spermatozoa motility indirectly by disrupting hormonal pathways or directly to the spermatozoa itself.

Exogenous antioxidant sources, such as vegetables, may become a solution to neutralize free radicals’ deleterious effects. Salam (also known as Indonesian bay leaf) leaf extract is known to have antioxidant activity and has been demonstrated to decrease blood glucose levels of diabetic rats [5].

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However, it is not known whether the antioxidant activity of salam leaf extract may also overcome the decline in the motility percentage of spermatozoa in ethanol-induced mice.

2. Materials and method

2.1. Animals
Male mice of DDY strain (Mus musculus L.) weighing 25–35 grams were used. Mice were kept in clean cages in a 12 h light/dark room. Mice were acclimatized for 14 days before using for experiments.

2.2. Salam (Syzygium polyanthum (Wight.) Walp.) leaves extract
Simplicia of salam leaves were purchased from BALITTRO (Balai Penelitian Tanaman Obat dan Aromatik) Bogor, West Java. A total of 250 g of simplicia was soaked in 70 % ethanol at a ratio of 1:10. Filtrates were obtained after 3 days, and then the residue was resoaked using the same solvent. The first and second filtrates were mixed. The filtrates were then evaporated using a rotary evaporator.

2.3. Experimental design
Mice were divided into 5 groups (n = 5 per group). The mice in control group 1 (KK1) were not treated. Those in control group 2 (KK2) were orally administered 0.5 % carboxymethylcelullose (CMC). Mice in treated groups 1 (KP1), 2 (KP2) and 3 (KP3) were orally treated with salam leaf extract (SLE) at doses of 43.75, 87.5 and 175 mg/kg body weight (bw), respectively. Group KK2, KP1, KP2, and KP3 mice were also given ethanol (40 % v/v) at a dose of 2.8 g/kg body weight (bw), 8 hours after induced by 0.5 % CMC and SLE. The experiment lasted for 20 days. The animals were sacrificed on the day following the last treatment using ether anesthesia and cervical dislocation. Sperms were collected from vas deferens. A pair of vas deferens were isolated and placed onto a watch glass containing 0.25 mL NaCl 0.9 %. Spermatozoa motility was calculated using the method employed for white blood cell counting in an improved Neubauer chamber.

2.4. Antioxidant test by qualitative DPPH method
SLEs were dissolved in methanol at a ratio of 1:10 and dripped onto a TLC plate. Vitamin E was used as a positive control, and methanol was used as a negative control. The TLC plate was then sprayed with a solution of DPPH in methanol. Antioxidant activity was studied on the basis of color change in the DPPH solution from purple to yellow.

3. Results and discussion
The data of motility percentage in the KK2 group (44.88 ± 3.40) based on least significant difference (LSD) test (P < 0.05) showed a significant difference compared with that in the KK1 group (58.62 ± 4.22) (figure 1). This indicates that decrease in the motility of spermatozoa treated with ethanol (40 % v/v) in mice dosed at 2.8 g/kg bw for 20 days has been observed.

The motility of spermatozoa is also affected by energy sources through the formation of ATP. During passage through the epididymis (maturation of spermatozoa in the epididymis), there is an increase in the carnitine levels in spermatozoa from caput to cauda. The carnitine is obtained from the luminal fluid of the epididymis. Carnitine serves as a shuttle to bring fatty acids into the mitochondrial matrix to produce acetyl co-A for the Krebs cycle to generate ATP [6]. The formation of the fluid content of the epididymal fluid is also strongly influenced by epididymal epithelial activity. As is well known, the function of epididymal epithelial cells is influenced by the hormones testosterone and DHT. Therefore, it is suspected that decreased testosterone, which is the substrate for DHT formation, might have negative affected epididymal epithelial function in this study [7].
A decrease in the percentage of motility occurring in the ethanol-induced mice (KK2) group might also be caused by disruption of spermatozoa storage conditions in the epididymis. Under normal conditions, mice spermatozoa are maintained in a quiescent state in the cauda epididymis and vas deferens. The storage conditions of the spermatozoa are governed by the pH value in the increasingly acidic lumen of the epididymis from kaput to cauda [7]. The acidic luminal epididymal fluid condition is regulated by clear cells actively secreting protons (H⁺) into the epididymal lumen [7, 8]. Regulation of epididymal epithelial cells, including the clear cells involved in regulating the pH conditions of the epididymal lumen fluids, is affected by DHT and estrogen hormones. Decreased DHT in the epididymis can cause decreased expression of estrogen receptors (Esr1 and Esr2) in epididymal epithelial cells. This disrupts ionic transport for luminal epididymis acidification. Thus, epididymal conditions become non-optimal for storage of spermatozoa [8].

Based on the LSD tests, the mice in KP1, KP2, and KP3 groups showed significantly higher percentage of spermatozoa motility than those in KK2 group. The percentage of motility in KP1, KP2, and KP3 were 56.97 % ± 2.66 %, 58.19 % ± 5.33 % and 53.77 % ± 4.00 %, respectively. This shows that SLE can maintain the quality of spermatozoa. Some of the contents of the bay leaves that might act as antioxidants include flavonoids, essential oils (eugenol), vitamin C, and vitamin E [9, 10]. Flavonoid compounds have antioxidant activity as free radical scavengers [3]. Scavenging activity possessed by compounds in bay leaves is thought to prevent damage caused by excessive free radical levels due to induction by ethanol.

Other compounds in bay leaves having antioxidant activity, in addition to phenolic compounds, are vitamins C and E. These vitamins possess antioxidant activity that can work synergistically with the glutathione antioxidant system. Both vitamins act by donating H⁺ to radical molecules in repairing cell membranes damaged because of free radical activity. Peroxyl radicals formed from lipid peroxidation in cell membranes are neutralized by the activity of vitamin E, thereby forming a radical vitamin E. Furthermore, vitamin E radicals are neutralized by vitamin C to form a radical vitamin C. Glutathione peroxidase (and phospholipid hydroperoxide glutathione peroxidase) further detoxify the oxidized fatty acids and repair the damaged membranes [11].
4. Conclusion
Based on the results of our research, it can be concluded that oral induction of ethanol (40 % v/v) at doses of 2.8 g/kg bw for 20 days can decrease the motility percentage of spermatozoa. Furthermore, the three doses of ethanol extract of bay leaf used in this study (43.75, 87.5 and 175 mg/kg bw) were proven to maintain the motility percentage of ethanol-induced spermatozoa in mice.

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