



## RESEARCH ARTICLE

# Increased levels of Follicle Stimulating Hormone (FSH) and sertoli cells count in wistar rats induced with paraquat after green bean sprout extract supplementation

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### ARTICLE INFO

### ABSTRACT

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Paraquat herbicide disrupts the hormone Follicle Stimulating Hormone (FSH), causes testicular damage and changes in spermatogenesis. This study aims to determine the effect of supplementation of green bean sprout extract (*Vigna radiata L.*) on FSH levels, Sertoli cell counts in male Wistar rats (*Rattus norvegicus*) induced by paraquat herbicide. This research uses true experimental with posttest only control group design. A total of 25 rats were divided into 5 groups: P0 was given standard feed, P1 was given paraquat 4 mg/g BW, P2 was given green bean sprout extract supplement (GBES) dose 21.6 mg/g BW and paraquat 4 mg/g BW P3 was given GBES dose 43.2 mg/g BW and paraquat is administered orally for 15 days. Measurement of FSH levels using ELISA, while Sertoli cells were observed using testicular histology prepare. Mann-Whitney test resulted significance of  $p < 0.05$ , which showed that the administration of GBES influenced FSH levels and the number of Sertoli cells. GBES doses of 21.6 mg/g BW, 43.2 mg/g BW, and 86.4 mg/g BW were shown to increase FSH levels and Sertoli cell counts in paraquat-induced Wistar rats. GBES increased FSH levels and the number of Sertoli cells in paraquat-induced rats.

## 1. Introduction

A person with infertility is incapable of achieving pregnancy after 12 months of unprotected sexual activity (Ismael *et al.*, 2017). According to a 2014 WHO report, 50% of male cases contribute to infertility worldwide (Yusoff *et al.*, 2017). Increased levels of reactive oxygen species (ROS) within the body cause male infertility (Durairajanayagam *et al.*, 2018). ROS affects communication between the testes in the hypothalamic-pituitary that disrupts the hormones (Durairajanayagam *et al.*, 2018). Pituitary Fails in Secreting *Follicle-stimulating hormone* (FSH) and *Luteinizing hormone* (LH) resulting in impairment of

testicular function (Ismael *et al.*, 2017). The increasing of ROS level can be induced by the paraquat, one of herbicide commonly used in the farming application.

The toxicity of paraquat administration orally dose 20 mg/kg BW in male mice, it is possible to reduce sperm quality (Anggraini *et al.*, 2018). Paraquat has the effect of changing the histological structure of the testes and causing spermatogenesis failure in male Wistar rats (Wasiu & Abdulfatai, 2019). Paraquat has the effect of changing the histological structure of the testes and causing spermatogenesis failure in male Wistar rats (Gawarammana & Buckley, 2011). So far, improving the male reproductive system due to exposure to paraquat is still not optimal, so the study of green bean sprouts

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(*Vigna radiata* L) which has antioxidant effects needs to be further studied.

Compared to non-germinated beans, green beans have higher levels of the antioxidant vitamins C and E (Zhai *et al.*, 2016). Vitamin E inhibits lipid peroxidation caused by free radicals via the free radical scavenger mechanism, specifically by donating one atom of its hydrogen ions to the peroxy radical to prevent fatty acid chain damage (Hidayat *et al.*, 2017). Antioxidants found in sprouts neutralize the pesticide paraquat by inhibiting the formation of free radicals, thereby preventing male infertility. Antioxidant vitamins E and C can influence the formation of FSH and LH by acting on the Hypothalamic-Pituitary-Testicular Axis (HHT). FSH is active on the development of spermatogonia to become a mature spermatid by going through receptors in sertoli cells then FSH also sangat determines the number of sertoli cells, so that both contribute to spermatogenesis. Vitamins E and C in the testes stimulate the growth of fine RE in leydig cells so that they can increase steroidogenesis; this demonstrates that both play a significant role in testosterone biosynthesis (Oduwole *et al.*, 2018; Prasad *et al.*, 2018). This study aims to determine the effect of supplementation of green bean sprout extract (GBES) on FSH hormone levels and Sertoli cell count in male Wistar rats (*Rattus norvegicus*).

## 2. Materials and Methods

The study was conducting using a true experimental design with only a post-test control group. The research was conducted with permission obtained from the Commission on Bioethics of Medical/Health Research, Faculty of Medicine, Universitas Islam Sultan Agung Semarang with Ethical Clearance No. 445/VII/2019/Bioethics Commission.

The paraquat herbicide used was Gramoxone 276 SL dose 200 mg/mL which was given to rats as much as 4 mg/g BW. 25 male Wistar strain rats aged 1-2 months and weighing 150-200 g were divided into five groups. P0 was fed with standard P1 received 4 mg/g BW of paraquat, P2 received 21.6 mg/g BW of green bean sprout extract supplement (GBES) and 4 mg/g BW of paraquat, P3 received 43.2 mg/g BW of GBES and 4 mg/g BW of paraquat, and P4 received 86.4 mg/g BW of GBES and 4 mg/g BW of paraquat. Oral administration is utilized for both GBES and paraquat. GBES was given on days 1-9, then on days 10, 11, 12, 13, 14, 15 were given paraquat herbicide treatment and GBES according to the treatment group. On the 16th day, rat blood collection was carried out intraocular to check FSH levels using the ELISA method (FSH Elisa Kit LS-F38325), then rats are terminated by cervical dislocation. To observe the number of Sertoli cells, rat testicles are histologically prepared using the

paraffin block method and Hematoxylin-Eosin staining after they have been surgically removed from male Wistar rats. Observation of the number of Sertoli cells as many as 5 fields of view by using the Olympus light microscope type CX41 with an Optilab camera magnification of 40×10.

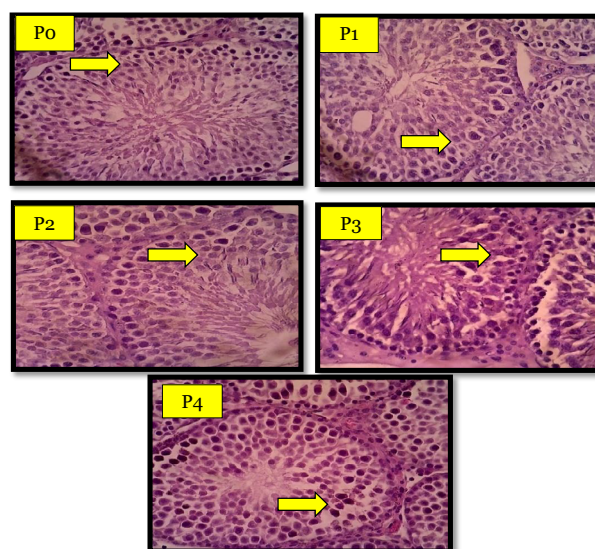
Data on FSH hormone levels and sertoli cell counts were analyzed using SPSS 21.0 software. The results of the Saphiro wilk normality test and the Levene homogeneity test indicated that the data on FSH levels and number of Wistar rat Sertoli cells in various treatment groups were not normally distributed and were not homogeneous ( $p < 0.05$ ), so the mean differences between groups were analyzed using the Kruskal Wallis non-parametric test followed by the uji Man Whitney test.

## 3. Results

Compared to the normal mouse group, rats exposed to paraquat doses of 4 mg/g BW for 15 days had significantly lower FSH levels and Sertoli cell counts ( $p < 0.05$ ). (P0). Supplementation with 21.6 mg/g

**Table 1.** FSH levels and sertoli cell counts of Wistar rats in various treatment groups

Group	FSH level (mIU/ml) (Mean ± SD)	Number of Sertoli cells (cells) (Mean ± SD)
P0	62,87±1,78	64,74±3.52
P1	20,36±0,69	28,98±3,04
P2	30,12±0,65	43,76±2,14
P3	40,77±1,54	51,69±1,16
P4	60,49±4,26	56,95±2,17



**Figure 1.** Histological overview of the testicles of Wistar strain rats in various treatment groups (Hematoxylin-Eosin staining, observations at magnification 40×10). Sertoli cells are indicated by a yellow arrow.

BW, 43.2 mg/g BW, and 86.4 mg/g BW of GBES can increase FSH levels and the number of Sertoli cells in rats exposed to paraquat 4 mg/g (groups P2, P3, and P4) (Table 1). Observations of histological preparations stained with H&E revealed that GBES improved testicular structure, as indicated by an increase in the number of Sertoli cells (Figure 1).

#### 4. Discussion

The purpose of this study was to determine the effect of the green bean sprout extract supplement (SEKKH) on FSH levels and the number of Wistar rat oil sert cells induced by the herbicide paraquat. The herbicide paraquat has the effect of increasing free radicals and reactive oxygen species (ROS) in the body, which damages the hypothalamic-pituitary-testicular axis's communication (HHT). Oral administration of paraquat at a dose of 20 mg / Kg BB can diminish the quality of spermatozoa in male rats (Anggraini *et al.*, 2018). In previous studies, paraquat has been shown to reduce spermatozoa quality, whereas in this study paraquat was able to reduce FSH levels and the number of sertoli cells in male Wistar mice, indicating that this study is consistent with previous research.

Increased ROS has a secondary effect of lipid peroxidation by causing mitochondrial damage to multiple cell lines. This decreases the effect of Hi on NADH ubiquinone oxidoreductase, resulting in an increase in Ca<sup>2+</sup> permeability across the mitochondrial membrane. Oxidation of NADPH also damages oxidative stress defenses, which are further focused on the activation of NF- $\kappa$ B, a proinflammatory cytokine (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6), and chemokines, nuclear condensation and DNA fragmentation, and apoptosis (Gawarammana *et al.*, 2011). Apoptosis reduces the viability of Sertoli cells as a result of an increase in caspase 3 secretion.

The presence of reactive oxygen species mediates sperm cell apoptosis by activating fas expression; fas is an indicator of apoptosis levels. The occurrence of apoptosis in spermatozoa can affect the number of spermatozoa, as indicated by an increase of up to 50 percent in the proportion of fas-positive spermatozoa (Mahdi *et al.*, 2012). Apoptosis is required to eliminate damaged sperm (Singh dan Agarwal, 2011). viability of Sertoli cells, which in turn determines the number of spermatozoa (Parekattil *et al.*, 2012; Oduwole, *et al.*, 2018).

Green bean sprout extract supplement (GBES) doses of 21,6 mg/g BW (P2), 43,2 mg/g BW (P3), and 86,4 mg/g BW (P4) influence the rise in FSH levels and the number of Sertoli cells. SEKKH possesses antioxidant properties that inhibit free radicals, thereby enhancing male reproduction. Mulyani (2016) found

that GBES doses of 108 mg/kg BW, 216 mg/kg BW, and mg/kg BW may increase the proportion of morphology and motility of spermatozoa in male mice exposed to Monosodium Glutamate (MSG) (Mulyani *et al.*, 2016).

Normal mice that received standard feed, namely in the P0 group, had significantly higher FSH levels and Sertoli cell counts when compared to groups P1, P2, P3, and P4. There is a correlation between dosage and time, if the dose of green bean sprout extract supplement is elevated and the time of more than 15 days is estimated to further increase FSH levels and Sertoli cell count. Antioxidant activity also affects, namely the P0 group has higher antioxidant activity when compared to the P1, P2, P3, and P4 groups so that it can reduce oxidative stress levels due to increased ROS levels in the body.

When there is an oxidation reaction, Vitamin C binds Vitamin E, which is oxidized free radicals, to form Vitamin E, which is reduced after obtaining hydrogen ions (H) from Vitamin C. This is how green bean sprout extract counteracts free radical (Prasad *et al.*, 2018). Vitamin E containing the group – CH donates H atoms to ROS, which protects PUFA by capturing peroxy radicals during the lipid peroxidation process so that the chain reaction can be broken and ROS levels in the body can be decreased.

By reducing ROS levels, antioxidants can repair testicular damage by enhancing sperm quality, thereby preventing infertility (Prasad *et al.*, 2018). These antioxidants influence the Hypothalamic-Pituitary-Testicular (HHT) axis, which controls the release of FSH and LH. FSH has a significant impact on the progression of spermatogenesis, so FSH levels also determine the number of sertoli cells. Vitamins E and C stimulate the development of fine RE in Leydig cells, thereby playing a role in testosterone biosynthesis (Oduwole *et al.*, 2018; Prasad *et al.*, 2018).

#### 5. Conclusions

Supplementation of green bean sprout extract at a dose of 21.6 mg/g BW, 43.2 mg/g BW, and 86.4 mg/g BW can increase in FSH levels and the number of Sertoli cells in paraquat-induced wistar rats

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