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Original Research Article

Curative Effects of Fruit Extract of Black Pepper (*Piper nigrum* L.) on the Testicular Function of Alloxan-Induced Hyperglycaemia Rats

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Abstract: Black pepper (*Piper nigrum* L.) is one of the most widely used spices which has many pharmacological effects and several health benefits. However, it is known to piperine of black pepper provide inconsistent effects on virility and fertility in male. In this study we aim the effect of black pepper fruit extract on testicular function—both hormonal and histological—in alloxan-induced hyperglycaemic rats. A total 24 male Sprague Dawley rats divided into four group (6 rats each). Group 1 (control) consisted of rats provided with food and water *ad libitum*. Group 2 consisted of rats induced with alloxan (150 mg/kg BW) on days 0 and 14 to serve as the hyperglycaemic group. Groups 3 and 4 consisted of hyperglycaemic rats administered black pepper extract every two days for 28 days at doses of 122.5 mg/kg BW and 245 mg/kg BW, respectively. The measured parameters included blood glucose levels, total testosterone levels, sperm quality (count, motility, and abnormalities), and testicular histological parameters (counts of type A spermatogonia, preleptotene spermatocytes, pachytene spermatocytes, spermatids, and Leydig cells). The results showed that hyperglycaemic rats treated with black pepper fruit extract exhibited reduced blood glucose levels, increased total serum testosterone, and improvements in sperm count, motility, and viability, alongside a decrease in sperm abnormalities. Histological analysis of the testes revealed an increase in the number of spermatogonia A, preleptotene spermatocytes, pachytene spermatocytes, and spermatids, as well as an increase in Leydig cells. Based on these data, it can be concluded that black pepper fruit extract has a curative effect on testicular dysfunction in alloxan-induced hyperglycaemic rats.

Keywords: Black Pepper, *Piper nigrum*, Testicular Function, Hyperglycaemia, Blood Glucose.

INTRODUCTION

Black pepper (*Piper nigrum* L) is a seed plant which is the main source of the natural ingredient piperine [1]. This plant is one of the most widely used spices in the world and has many pharmacological effects and several health benefits. Among the reported pharmacological benefits of piperine are: antioxidant, anti-inflammatory, anti-diabetes, anti-obesity, anti-arthritis; and provide therapeutic benefits in patients suffering from oral and breast cancer, metabolic syndrome, hypertension, cerebral stroke, cardiovascular diseases, and kidney diseases [2, 3].

Despite its numerous health benefits, piperine, particularly from black pepper, is known to provide inconsistent effects on virility and fertility in men. In 1999, Malini and colleagues reported that in mature albino rats piperine at a dose of 10 mg/kg BW caused reduction in testis weight and accessory sex organ, caused severe damage to the seminiferous tubules, decreased in Leydig cells diameter [4].

However, this substance is also known to stimulate Leydig cells, enhancing testosterone levels that contributing to improved erectile function [5]. Testicular function naturally influences male sexual behaviour. The effect of black pepper fruit extract on sexual drive of males has been reported by Sutyarso *et al*., (2015). The report reveals that the administration

of black pepper fruit extract increases sexual drive of male mice, as evidenced by a shorter courtship latency during mating tests [6].

Subsequently, in 2016, Sutyarso and colleagues reported that the administration of black pepper extract significantly increased serum testosterone levels and enhanced epididymal tubule weight, epididymal sperm concentration, spermatocyte and spermatid counts. [7], Nevertheless, research conducted by Chen *et al.,* (2018) found that piperine is known to significantly reduce fertility in males due to the inhibition of spermatogenesis [8].

Other studies indicate that low to moderate doses of black pepper extract significantly reduce sperm motility and viability while increasing sperm abnormalities. Interestingly, high doses show a restorative effect on sperm count and motility, although abnormality levels remain high [9]. Previous research has shown similar results, where piperine restored male fertility parameters to normal levels after treatment was discontinued, suggesting its potential use as a reversible contraceptive [10].

Based on the data above, it can be asserted that many questions remain regarding the effects of piperine found in black pepper fruit on male reproductive and sexual function. Hyperglycaemia is one of the factors contributing to a decline in male reproductive health. Various studies have shown that hyperglycaemic conditions can be induced by alloxan [11, 12].

Therefore, in this study, we aim to further investigate the effects of black pepper fruit extract administration on testiculare function—both hormonal and histological—in alloxan-induced hyperglycaemic rats.

MATERIALS AND METHODS

Plant Material and Extraction

Mature-fresh black pepper fruit was collected from a farmer in Ngarip Village, Ulubelu District, Tanggamus Regency, Lampung Province. Its drupe then grounded into fine flour. To make the fruit extract then the drupe powder was soaked in 95% ethanol at room temperature. For three days the supernatant was collected every 24 h and filtered to remove impurity components. Next, the filtrate was concentrated in a rotary evaporator at a temperature of 40 °C and a pressure of 60 mbar. The extract is stored in the refrigerator as stock until used.

Animals and Experimental Designs

Male Sprague Dawley rats obtained from the animal house at the IPB University, Bogor, Indonesia. The total of 24 rats, aged 10-12 weeks, weight 180-200g, were grouped into four based on the treatment given: control, hyperglycaemia control and pepper fruit extract group. The control group is the rats that only received water and food *ad libitum.* The hyperglycaemia control group is the animals treated only with alloxan intraperitoneally on day 0 and day 14 at a dose of 150 mg/kg BW. While the treatment group is the rats received alloxan (day 0 and day 14) and given black pepper fruit extract every two days for 28 days at a dose of 122.5 mg/kg BW (Group 3) or 245 mg/kg BW (Group 4). All procedures for animal caring are in accordance with the guidelines of the Health Research Ethics Commission of the Faculty of Medicine, Lampung University (No.3468/UN26.18/PP.05.02.00/2024).

Blood Glucose Assessment

To determine the blood sugar levels of the animals, a pericardiocentesis method was used. On day 29, the rats were anesthetized and blood was drawn directly from the heart using a sterile syringe. By using ABX Pentra Glucose HK CP (Horiba, Japan) the blood glucose concentration was assessed at a wave length of 500 nm. The blood glucose concentration is expressed in mg/dl.

Testosterone Assay

Blood samples were collected from the orbital plexus using a haematocrit pipette, transferred to separate test tubes, and centrifuged at 4,000 RPM for approximately 20 minutes to separate the serum; the tubes containing the serum were subsequently stored at -20°C until required for analysis. Serum testosterone levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit from Rodent Testosterone Endocrine Technologies Inc., which has an analytical sensitivity of 0.06 to 0.07 ng/ml and a standard quantitative working range of up to 25 ng/mL.

Sperm Examination

To studied testis histology the rats were terminated by injecting ketamine at a dose of 100 mg/kg BW and cervical dislocation. Right testes and cauda epididymis of the animals were removed using a dissecting kit. The cauda epididymis was pinched to squeeze the spermatozoa. Spermatozoa suspension was made by mixing the samples with NaCl 0.9%. The spermatozoa were then counted using a Neubauer's haemocytometer under a light microscope (400x) and expressed as millions/ml. Sperm motility was evaluated by counting motile and immotile sperms. The normality of sperm was observed after the object dried under a Richter Optica HS-3B-3's light microscope (China) at 400x magnification.

Testis Histology Examination

Testicular histology examined using Haematoxylin & Eosin (HE) staining. Spermatogonia and spermatocyte were counted in 10 random seminiferous tubule cross-sections per testis. Leydig cells were counted in 10 non-overlapping interstitial fields with thickness of 4–5 µm. The count was normalized to area using National Institute of Health Image J software version 1.8.0 (New York, USA) at 400x magnification [13].

Statistical Analysis

All data are presented as mean values and analysed using one-way ANOVA. The ANOVA results are further analysed using the Tukey post hoc test in SPSS version 27.

RESULT AND DISCUSSION

Blood Glucose

The results of examining the blood glucose levels of rats from all treatment groups are presented in Figure 1. Administration of black pepper fruit extract at a concentration of 245 mg/kg BW was shown to reduce blood glucose levels in alloxan-induced rats ($p<0.001$). Although the levels remained higher than those of normal rats, the difference was not statistically significant ($p=0.403$).

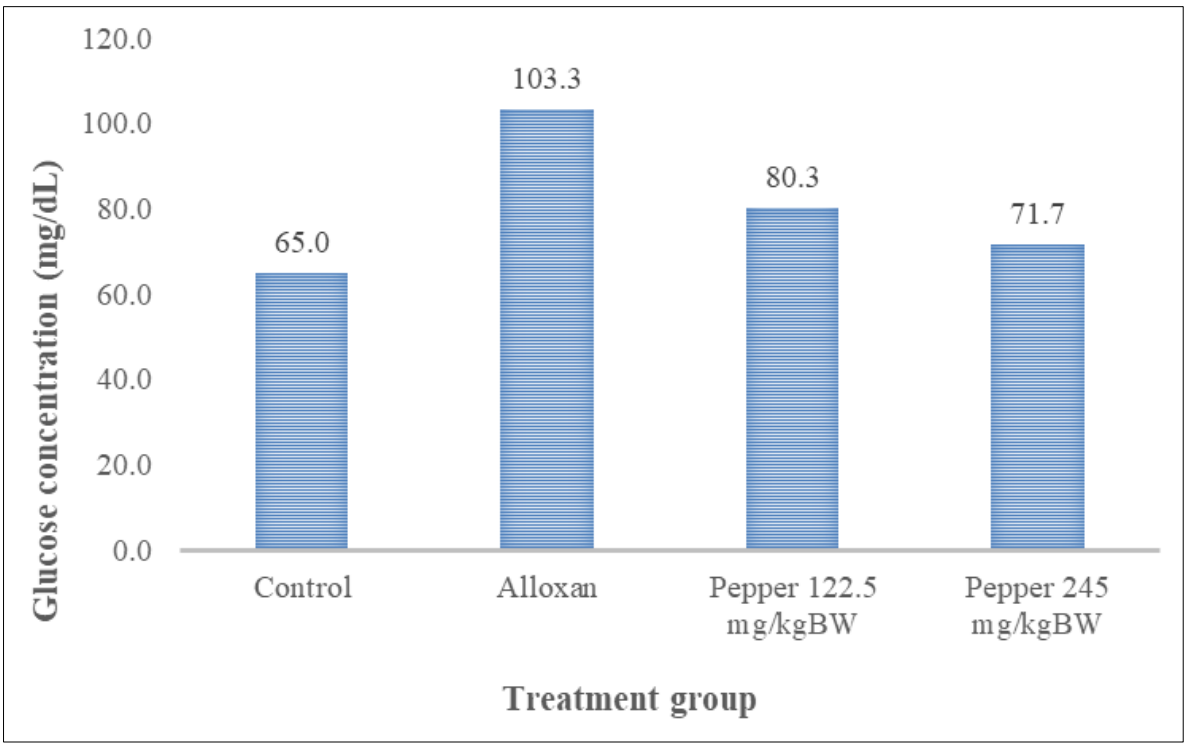

Figure 1: Effect of black pepper fruit extract on the blood glucose concentration

Previous research has also identified the antihyperglycemic property of piperine, showing that subacute administration tends to lower blood glucose levels [14]. This is thought to be because piperine induces the phosphorylation of AMP-activated protein kinase (AMPK) and its downstream target, acetyl-CoA carboxylase (ACC) [15]. It is also known that piperine can ameliorate pancreatic β-cell dysfunction, thereby increasing glucose uptake by the pancreas [16, 17].

Total Serum Testosterone

Figure 2 presents the effect of black pepper fruit extract administration across all treatment groups. The figure 2 clearly shows that alloxan induction significantly reduced total serum testosterone levels ($p<0.01$). However, the administration of black pepper fruit extract significantly increased testosterone levels—even surpassing those of normal rats—although the difference was not statistically significant.

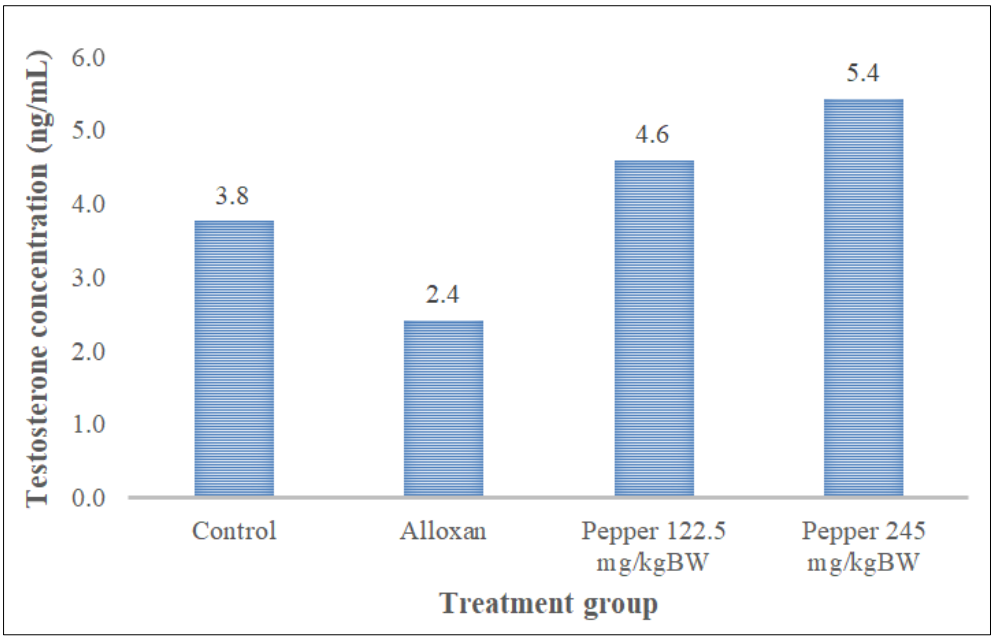

Figure 2: Effect of black pepper fruit extract on the total serum testosterone concentration

Piperine is a potential inhibitor of testosterone 5α-reductase; by inhibiting this enzyme, the conversion of testosterone to dihydrotestosterone (DHT) can be prevented, thereby maintaining high serum testosterone levels [18]. Another finding of this study is that pepper fruit extract increases the number of Leydig cells in alloxan-induced hyperglycemic rats. Leydig cells produce testosterone through the binding of luteinizing hormone (LH), which activates a G-protein-coupled pathway, thereby triggering an increase in cyclic adenosine monophosphate (cAMP) and activating protein kinase A (PKA) [19].

Sperm Parameter

The effects of black pepper fruit extract administration on rat sperm parameters are presented in Table 1. The data reveal that black pepper extracts increased sperm count in hyperglycaemic rats to levels comparable to those of normal rats ($p=0.026$). Sperm motility was enhanced to a level exceeding that of normal rats ($p=0.014$). Furthermore, sperm viability was restored by the extract to a level surpassing that of normal rats, while sperm abnormality was reduced to levels comparable to those of normal rats.

Table 1: Effect of black pepper fruit extract on number and quality of sperm in rats

Treatment Group	Sperm Count (x10^6)	Sperm Motility (%)	Sperm Viability (%)	Sperm Abnormal (%)
Control	45.35±6.07^b	70.05±3.36^b	78.1±6.19^b	12.8±3.25^a
Alloxan	19.55±2.75^a	38.457±2.93^a	67.36±7.32^a	28.91±6.81^c
Pepper 122,5 mg/kgBW	42.01±3.78^b	76.31±3.71^c	79.68±4.29^b	16.36±5.26^{ab}
Pepper 245 mg/kgBW	39.76±5.19^b	77.69±3.01^c	78.18±6.34^b	13.82±2.44^{ab}
Values are presented as mean ± SD; values in the same column followed by the same superscript are not different statistically.				

The results of this study clearly indicate that the administration of black pepper fruit extract to hyperglycaemic mice tends to increase sperm count and quality. A decline in sperm count and quality is caused by oxidative stress. Oxidative stress can significantly impair sperm count and quality—including morphology, motility, and DNA integrity [20]. This may be attributed to the antioxidant properties of piperine, which exhibits the most potent DPPH (2,2-difenil-1-pikrilhidrazil) radical scavenging activity [21].

Testis Histology Parameter

Effects of fruit extract of black pepper on the rat testicular histology were presented in Tabel 2. Data from the table reveal that black pepper fruit extract successfully increased the numbers of type A spermatogonia ($p=0.031$), preleptotene spermatocytes ($p=0.017$), pachytene spermatocytes ($p=0.029$), spermatids ($p=0.012$), and Leydig cells ($p=0.011$). Interestingly, these levels even exceeded those observed in normal rats.

Tabel 2: Effect of black pepper fruit extract on testicular histiology in rats

Treatment Group	Spermatogonia A	Preleptotene Spermatocytes	Pachytene Spermatocytes	Spermatids	Leydig Cells
Control	7.55±1.86^{ab}	20.13±3.38^{b}	46.51±8.6^{b}	113.33±8.36^{b}	56.61±8.58^{ab}
Alloxan	7.65±1.45^{a}	16.03±4.21^{a}	24.61±8.00^{a}	75.07±8.53^{a}	47.04±9.10^{a}
Pepper 122.5 mg.kgBW	8.16±1.03^{b}	22.68±3.72^{b}	49.59±8.21^{b}	121.91±15.95^{bc}	60.82±7.25^{b}
Pepper 245 mg/kgBW	7.8±1.62^{ab}	20.76±2.07^{b}	47.38±10.36^{b}	123.09±10.81^{bc}	62.07±5.01^{b}
Values are presented as mean ± SD; values in the same column followed by the same superscript are not different statistically.					

Based on the data in Table 2, it is evident that the administration of black pepper extract to hyperglycemic rats improved testicular histological parameters. This may be associated with the high testosterone levels observed in the rats treated with black pepper extract (Figure 2). Testosterone is known to be essential for the initiation of spermatogenesis. The absence or imbalance of hormones within the hypothalamic–pituitary–gonadal (HPG) axis can lead to disruptions in spermatogenesis [22, 23].

CONCLUSION

Administration of black pepper fruit extract to alloxan-induced hyperglycaemic rats resulted in reduced blood glucose levels, increased testosterone levels, and improvements in sperm count and quality as well as testicular histology. Therefore, it can be concluded that black pepper fruit extract has a curative effect on male testicular dysfunction.

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