THE EFFECTS OF PHYLLANTHUS GOMPHOCARPUS HOOK. F. AQUEOUS EXTRACT SUPPLEMENTATION ON ANDROGENIC HORMONAL LEVEL AND HISTOLOGICAL TESTES MORPHOMETRY

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Graphical abstract

Abstract

Phyllanthus gomphocarpus Hook. F is a herb found in tropical rainforest countries and has been widely used in traditional medicine especially in Peninsular Malaysia to treat male infertility problems and increase human health. Ten percent aqueous extraction of PGR was prepared by heating in 40º C for 12 hours and its decoction were freeze dried to obtain crude extract. Total numbers of 24 male rats were randomly divided into 4 groups which NEC, POC, PGR50 and PGR800. Except for NEC group, other groups were induced genitotoxicity by oral supplementation of 200 mg/kg of BPA for 21 consecutive days. For PGR50 and PGR800 groups, the animals were concomitantly supplemented with 50 mg/kg and 800 mg/kg of PGR extracts respectively. Blood and testes sample were collected for hormonal and testes morphometric analysis. Results clearly showed that testosterone (T), the most important hormone in spermatogenesis process were significantly increased in PGR supplemented groups (p<0.05) compared to NEC and POC groups. For the level of DHT, FSH, and LH hormonal level, the results were varies and can be explained with the deterioration effects of BPA and the hormonal regulations itself. Testes morphometric study showed no significant difference in the seminiferous tubule diameter in each groups but was significantly increased (p<0.05) in the epithelium height, which indicates higher rate of spermatogenesis process. In addition, PGR possessed a protective effect against the toxicity effects of BPA. In conclusion, PGR was suggested to enhance male fertility through improvement of androgenic hormones and possessed a protective effect against genitotoxicity of BPA.

Keywords: Phyllanthus gomphocarpus Hook. F, Bisphenol A (BPA), androgenic hormones, testes morphometric, spermatogenesis

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1.0 INTRODUCTION

Phyllanthus gomphocarpus Hook. F, is a herb found in tropical rainforest countries and has been widely used in traditional medicine especially in Peninsular Malaysia. This plant belongs to the Phyllanthaceae family and known as Cermela Hutan as their local name. To date, the scientific data about the beneficial effects of this plant is still lacking. However, other plants from its family, Phyllanthaceae, have been identified to contain numerous beneficial effects towards human health such as antimicrobial, anti cancer, anti diuretics, anti – inflammatory, inhibits cytotoxicity and genotoxicity activities, antioxidant, anti inflammatory and also anti diabetics [1]–[3].

Male infertility was associated with androgenic hormone-related diseases, such as varicocele,
cryptorchidism, testicular cancer and cardiovascular diseases [4], [5]. Normal testicular function is to synthesis testosterone which needed not only for sperm production, but also for the development of secondary male sexual characteristics and also normal male behavior [6]. Testicular injury especially to the male germ cells, such as Sertoli cells and Leydig cells is also one of the major causes of infertility. It was due to the compensatory increase in gonadotropins, which overcomes minor defects in testicular function to synthesize testosterone leading to spermatogenesis disruption [7]. Besides that, tumor on the pituitary gland causes Luteinizing Hormone (LH) and Follicle Stimulating hormone (FSH) fails to be synthesized and cause androgenic imbalance as well as impaired the sperm production [8].

Androgenic hormonal imbalance may be due to the environmental factors that reduce the sexual hormone production in male as well as their fertility rates. Bisphenol A (BPA), which is widely used in the chemical industry in the manufacturing of epoxy, polycarbonate and polyester-styrene resins, and in dentistry, is one of the environmental factors that contributes to male infertility [9]. BPA also was reported to react like the female hormone by binding to the estrogen receptors in vitro and in vivo [10] and promoted as a synthetic estrogen (xenoestrogen) [11]. Like estrogen, xenoestrogen affect the spermatogenesis process by two mechanisms; suppressing testosterone production by directly impaired the Leydig cells or through negative feedback on gonadotrophin releasing hormone secretion [5]. Besides that, BPA also found to affect male fertility by producing remarkable degenerative changes in the epithelium structure of the testes after several times of exposure, even at low doses [12]. Therefore, clinical evaluation of androgen factors that may contribute to male infertility should be considered.

In current American Urological Association (AUA) guidelines, androgenic hormones measurements should be performed at least on the two most important male hormones which are serum FSH levels and serum testosterone levels in the patients suspected of infertility [13]. This is because the testosterone was considered as the indicator for endocrine balance and normal functioning of Leydig cells, whereas FSH level is thought to be a representative parameter of spermatogenesis process [14]. Testicular deficiency evaluation usually present with high level of FSH and LH, and sometimes with low level of testosterone. This condition occurred because the level FSH was correlate with the number of spermatogonia which when absent or diminished, FSH level usually elevated [15]. However FSH result alone does not accurately predict the spermatogenesis process but the other androgenic hormones are also important to be measured as ways to detect the roots of the male infertility problem for an individual.

In the other hand, testicular morphometry is one of the quantitative method in assessing changes in testicular tissue and spermatogenic functions from various factors [16]. Morphometric information is valuable for the correlations with the other physiological findings to briefly understand spermatogenesis process by assessing changes on the seminiferous tubule structure [17]. Thus, this study was sought to determine the effects of Phyllanthus gomphocarpus Hook F. roots supplementation on the hormonal changes by assessing their androgenic hormones and their testicular morphometric from the histological section.

2.0 MATERIAL AND METHOD

2.1 Plant Identification

Three kilograms of fresh roots of Phyllanthus gomphocarpus Hook. F. (PGR) was collected from Felda Keratong, Pahang Darul Makmur, Malaysia. The whole part of the plant were collected for identification and verification at the Rimba Ilmu Botanical Garden, University of Malaya, Malaysia and was deposited in the herbarium unit with voucher number of KLU 47925.

2.2 Preparation of PGR Extract

The roots were cleaned, sliced into small pieces and dried in a hot air oven at 40°C for approximately 1 week. The dried materials were ground into powder and kept in air tight container before used. Ten percent (10%) PGR aqueous extraction was prepared by mixing 100g of pulverized root into 1L of distilled water and incubated in 40°C of temperature for 12 hours. Then the mixture was filtered and the filtrate was freeze dried to obtain powdered PGR extract.

2.3 Animals Experimental Protocol

Twenty four male Wistar albino rats were purchased from Laboratory Animal and Facilities Management (LAFAM), Universiti Teknologi Mara (UiTM), Malaysia and maintained at controlled conditions of temperature (24 - 27°C), equal light-dark cycle, open ventilation and were fed ad libitum. After acclimatization for one week, rats were randomly divided into four groups (n=6) and supplemented orally with bisphenol A (BPA) and different PGR aqueous extract concentration for 21 days. Negative Control (NEC) group was administered orally with 2 ml of distilled water and Positive Control (POC) administered orally with 1 ml of 200 mg/kg of BPA together with 1 ml of distilled water as placebo. For PGR 50 and PGR 800 experimental group, rats were supplemented orally with 1 ml of 200 mg/kg of BPA together with 50 mg/kg and 800 mg/kg of PGR aqueous extract respectively. The experiments were
endorsed by UiTM Committee of Animal Research & Ethics (UiTM CARE : 71/2015).

2.4 Blood Sample Collection

After 21 days of treatment rats were anesthetized and about 5 ml of blood collected into plain tube by cardiac puncture technique. The blood was allowed to clot for 30 minutes and then centrifuged 3500 rpm for 10 minutes to separate between serum and red blood cells (RBC). The serum then aliquoted equally into 4 different 1.5 ml bullet tubes labeled as FSH, LH, DHT and T before kept in -80°C C freezer prior to use.

2.5 Blood Sample Preparation and Hormonal Analysis

Frozen sera were thawed several hours before the hormonal analysis performed which includes FSH, LH, DHT and T hormonal assay using Enzyme Linked Immunosorbtant Assay (ELISA) assay kits (Cusabio, China).

2.6 Testes Sample Collection and Preparation

Histology analysis was performed by using Mayer’s Haematoxylin and Eosin (H&E) staining method. The testes were grossed transversely in about 3 – 5 mm thick and placed into a tissue cassette for processing which encompasses of dehydration, clearing, infiltration and impregnation of tissue by using a complete series of chemicals including xylene, multiple percentages of alcohols as well as paraffin wax. After that, the tissues were embedded to form a tissue block, before sectioning using a microtome. Ribbon of the tissue block from the sectioning process were picked onto a clean glass slide and dried on a hot oven. The slides were stained by H&E staining and mounting prior to analysis.

2.7 Testes Morphometric

Morphometric measurements were performed using Leica DM LB light microscope, equipped with a colour CCD video camera (Leica DC200) to capture the image of the histological testes under lower magnification (10X). Twenty round or nearly round tubular profiles with a clear lumen were selected randomly and their diameter was measured for each section by using Motic Image Advanced 3.2 program. The same tubules were used for the measurement of seminiferous epithelial height from the basement membrane to surface of the epithelium and the average of four diametrically opposed measurements were taken for each section [18]–[20].

2.8 Statistical Analysis

All data were analyzed using Statistical Package for Social Science (SPSS) program version 21. The significance difference between means was established by subjecting the results to analysis of variance (ANOVA) followed by post hoc Tukey test. All data are presented as average ± standard deviation (SD) and shown in figure 1 and Figure 2. Values of p<0.05 were denoted as statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Testosterone Level

Male infertility such as loss of libido, impotence, oligospermia or azoospermia may cause by hypogonadism, a deficiency in the secretion of male gonadal hormones. In this current study, testosterone (T), dihydrotestosterone (DHT), follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured from the serum sample of Phyllanthus gomphocarpus root extract (PGR) supplemented rats. The effects of the supplementation toward male fertility were assessed by these male androgenic hormonal changes because all of the hormones played a major role in male reproductive systems especially spermatogenesis process [5] whereas testosterone was the most important androgenic hormone to stimulate and maintain the spermatogenesis process [21]. From the results obtained from Table 1, the level of testosterone from both PGR50 and PGR800 groups were significantly increased when compared to the NEC and POC groups (p<0.05). The increased level of testosterone in these groups suggested that the plant possessed a fertility enhancing effects in rat model. The same finding was described by Zanoli et al., (2009) where extracts from Eurycoma longifolia Jack (Tongkat Ali) was found to improved male sexual function especially in the copulatory behavior via higher level of testosterone stimulated. Comparing to the normal level of testosterone from the NEC group, it can be described that PGR supplementation could improve male sexual function through facilitating higher level of testosterone production. The finding also in consistent with other studies that showed herbal origin intervention such as Eurycoma longifolia, Alpinia calcarata Roscoe, Carinella sinensis, Mondia whitei and Zingiber officinale have the ability to increase the level of testosterone as well as naturally improve the male infertility problem [23], [24].

3.2 Dihydrotestosterone (DHT) Level

Other than testosterone, dihydrotestosteron (DHT) also been measured in this study. DHT is a testosterone-derivative hormone synthesized by 5 alpha-reductase enzyme that play important role to support spermatogenesis, especially in low testosterone level condition and also important for the development and the protection for the prostate [30], [31]. In this study, the level of DHT were significantly increased in both PGR supplementation
groups compared to NEC group (P<0.05). This findings was consistent with a study performed by Lewis et al., (2002), that reported isoflavone extract could increase the level of DHT, protective the prostate and improve male sexual functions [30]. Since DHT is important during spermatogenesis and prostate protection, the findings suggested that PGR could give beneficial effects on male reproductive system through increasing the level of DHT. However, DHT level in POC group also significantly increased when compared to NEC and PGR50 groups (P<0.05).

This situation may be explained by the effects of BPA on the prostate tissue. It was found to express an androgen receptor mutation that may lead to the prostate cancer and also can compete with DHT for the binding with androgen receptors [32]. As discussed before, DHT mainly found in the target tissue especially the prostate tissue. Thus, the genitotoxicity effects of BPA on the prostate tissue can be the main reason for the instability of the DHT production and regulation.

Nevertheless, PGR supplementation seems provide the potential to recover the effect of BPA against prostate tissue and DHT regulation. It can be proved by the level of DHT in PGR50 was significantly lower compared to POC group but found to be significantly higher when compared to NEC group. It was suggested that PGR supplementation could decrease the unforeseen level of DHT, induced by the BPA.

3.3 Luteinizing Hormone (LH) Level

Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) were secreted by the anterior pituitary gland in spermatogenesis process. The result Table 1 showed that the level of LH was significantly higher in NEC group and significantly lower in POC group (p<0.05). The higher concentration of BPA administered in this study was the main reason for the decreased level of circulating LH level in POC. PGR50 and PGR800 groups compared to NEC group. Study by Gharravi et al., (2006) found that BPA supplementation on male rats disrupt normal synthesis of LH production in pituitary gland by decreasing the level after several period of trials [25]. However, PGR supplementation in both groups showed that, the toxic effects of BPA on LH level was reduced by significantly increased in the level of LH compared to POC group (p<0.05). The same finding was revealed where supplementation of Zingiber officinale increased LH level in 75 infertile men and hence suggesting herbal intervention play an important role to treat infertility problem in vivo [26]. Although the level of LH was increased in PGR supplemented groups, the level was not comparable to the NEC group. Negative feedback of androgenic hormonal regulation could possibly be the underlying mechanism that explained on this result. When adequate or high level of testosterone secretion by Leydig cell was achieved, the pituitary gland will be stimulated to suppress the secretion of LH [21], [27].

3.4 Follicle Stimulating Hormone (FSH) Level

On the other hand, the level of FSH was found to be high in POC group and low in the PGR50 group (p<0.05). The data was consistent with a cross sectional study which demonstrated that the level of FSH was significantly increased in the paint workers exposed to the BPA compared to the normal control group [28]. Similarly, supplementation of PGR in this study causes a reduction of BPA intoxication and hence lowering the level of FSH towards normal, as represented by the NEC group. This finding was in line with a study performed on Tualang honey and its effects on the female hormonal regulations. They proved that Tualang honey supplemented with BPA could hinder the toxicity effects of the BPA against normal hormonal cycle by decreasing the elevated FSH level induced by BPA alone [29]. Since FSH is important in stimulating the spermatogenesis process [30], it was clearly indicated that PGR could enhance and protect male reproductive systems through the stabilization of androgenic hormonal regulations.

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<th>PARAMETERS</th>
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<td>NEC</td>
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<td>Follicle Stimulating Hormone (FSH)</td>
<td>0.63 ± 0.02 mIU/ml³</td>
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<tr>
<td>Luteinizing Hormone (LH)</td>
<td>0.79 ± 0.02 mIU/ml³</td>
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<tr>
<td>Testosterone Hormone (T)</td>
<td>28.77 ± 6.01 ng/ml³</td>
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<tr>
<td>Dihydrotestosterone (DHT)</td>
<td>461.76 ± 93.63 pg/ml³</td>
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3.5 Seminiferous Tubule Diameter

The testis is a heterogeneous organ, which contains seminiferous tubules where the spermatogenesis takes place, and the interstitium, the space between the tubules, where endocrine function to regulate spermatogenesis [31]. In this study the morphometric evaluation of the testes histological sections were evaluated by measuring the seminiferous tubule diameter (STD) and the seminiferous tubule epithelial height (SEH). Results from this study showed that STD in PGR800 group was significantly higher compared to NEC group (p<0.05) suggesting Phyllanthus gomphocarpus Hook roots extract supplementation facilitates the higher rate of spermatogenesis process [20]. This finding was in contrast with a result obtained from a previous study reported that stanozolol decreased STD and affect the smoothness of spermatogenesis hence delay sperm production in Sprague-Dawley rats [32].

3.6 Seminiferous Epithelial Height

Seminiferous tubule epithelial height (SEH), strictly related to the spermatogenesis process occurred in the testes. Changes in the SEH parameter could reflect the level of sperm produces in the testes [18]. From the results, POC group which supplemented with BPA alone showed a significantly decreased of SEH level compared to the other groups of treatments (p<0.05). This finding was in agreement with a previous study performed by Gurmeet et al., (2013) that revealed BPA disrupts spermatogenesis process by decreasing the level of SEH [33]. In contrary, BPA toxicity effect was neutralized by PGR supplementation indicated with an increased SEH level. Previous study reported that Tribulus terrestris facilitates the same mechanism as PGR by increasing the SEH measurement after BPA induction for 8 weeks [34]. The overall data may not conclude on the protective effect of Phyllanthus gomphocarpus roots extract in male reproductive system but findings from the study and similarity of results from previous reports may suggest on the beneficial effect of herbs intervention such as Phyllanthus gomphocarpus on the improvement of male infertility problem hence further investigation on the herbs is suggested.

4.0 CONCLUSION

This study demonstrated that PGR possessed beneficial effects on male reproductive system and the effect was through the increased of the androgenic hormones regulation especially testosterone. Improved testosterone level facilitates the rate of spermatogenesis and increase seminiferous epithelium height. Interestingly, PGR also possessed the protective effects against the deterioration effects of BPA on the androgenic hormonal productions and the structure of testes seminiferous tubules, as well as spermatogenesis process. Thus, PGR may enhance male fertility through the increasing number of androgenic hormones and hence have the potential to be explored further for the benefit towards human health.

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References


