ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 12, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

TRANSFORMING GROWTH FACTOR BETA OF TESTIS GERMINAL CELL IN GUINEA PIG (*CAVIA PORCELLUS*) AFTER EXPOSURE TO METHANOL EXTRACT OF THE SEEDS OF BITTER MELON (*MOMORDICA CHARANTIA*) AND DEPOT MEDROXYPROGESTERONE ACETATE

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Received: 12 June 2018, Revised and Accepted: 23 July 2018

ABSTRACT

Objective: The discovery of male contraceptive drugs continues to be pursued, due to the very small participation of men associated with the lack of contraceptiveÐptions for men. The combination of methanol extract of bitter melon seed and depot medroxyprogesterone acetate (DMPA) becomes the choice that currently being pursued to be applied to men.

Methods: The use of guinea pigs as experimental animals conducted research using experimental methods with complete randomized design. The study was divided into four control groups i.e K0; dimethylsulfoxide (DMSO) for 0 week (4 h),K1; Control group of DMSO for 4 weeks, K2; Control group of DMSO for 8 weeks, K3; Control group of DMSO for 12 weeks, and four treatment groups, i.e: group P0; bitter melon seed extract of 50 mg/100g body weight/day for 0 week (4 h), group P1; Bitter melon seed extract of 50 mg/100g BW/day for 4 weeks+DMPA, group P2; Bitter melon seed extract of 50 mg/100g BW/day for 8 weeks+DMPA, P3 group; Bitter melon seed extract of 50 mg/100g BW/day for 12 weeks+DMPA.

Results: There was a significant effect ($p<0.05$) methanol extract of bitter melon seed to increase the transforming growth factor expression -β expression.

Conclusion: The methanol extract of bitter melon seed was able to be candidate for herbal contraception.

Keywords: Testis histology, MDA, Bitter melon, Guinea pig.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i12.27920

INTRODUCTION

According to the Survey of Indonesia Health Demographic at the 2002, family planning participation is still very low, only 4.4%, which include condom use (0.9%), vasectomy/male surgery method (0.4%), intermittent intercourse (1.5%), and periodic abstinence (1.6%) [1]. The participation rate as a family planning acceptor is still very low when compared with Islamic countries, such as Bangladesh at 13.9% in 1997 [2] and Malaysia at 16.8% in 1998 [3,4].

Male was less interested in becoming "family planning" acceptor because there were no many contraceptive options available [5-7]. Therefore, it is necessary to develop from herbs, one of the seeds of bitter melon (*Momordica charantia*) [5-18]. Combination of bitter melon seed with depot medroxyprogesterone acetate (DMPA) was better able to suppress spermatogenesis in mice [19,20] and rabbits so as to decrease the quantity and quality [21]. The content of flavonoids in bitter melon seeds can lower serum testosterone levels so that it can cause decreased libido [22-24]. Therefore, DMPA should be added which is the source of testosterone in serum [1,19,20,25-31].

But in chemical compound in plant, Highly variable toxicities in plants and tissue sensitivity, depending upon the solvent used for extraction, the tool likes Column chromatography of the crude extracts lead to a number of fractions such as as potent anti cancer agents, scopoletin and β-sitosterol glucoside those have antioxidant property [127-130].

The workings of bitter melon seed extract and DMPA through hormonal mechanism of hypothalamus-pituitary-testis like assayed adrenocorticotropic hormone, cortisol and prolactin and activate of HPA axis [32-47]. Decreased intratesticular testosterone levels can cause disruption to spermatogenesis so that the resulting spermatozoa is reduced even cannot be produced. Testosterone may enter the seminiferous tubule and bind by androgen-binding protein (ABP) so that it can be used for the growth and development of spermatogonia as its stem cell spermatozoa [48-54]. Testosterone is required when spermatogonia are transformed into spermatocytes, spermatids, and spermatozoa in the seminiferous tubules [34,44,55,56].

Transforming growth factor beta (TGF-β) is a protein secreted to regulate the proliferation, differentiation, and death of different cell types [57-66]. If linked to the research of bittter melon seed extract and DMPA, then there is no report that reveals in detail about TGF-β picture in tubulus seminiferus after giving extract methanol bitter melon seed and DMPA. Therefore, this study is very important to explain the existence of TGF-β in spermatogenesis in seminiferous tubules.

METHODS

The study subjects used healthy male healthy guinea pigs aged 8–11 months (proven to have once-a-year offspring when mated with a female guinea pig) weighing 400–450 g and placed in a clean cage. The study has received permission from the Research Ethics Committee of Health with no. 085/KEPH-FMIPA/2017.

Measurement of phytochemistry

Fenol test

Phenolic test was performed by reacting leaf ethanol extract and mulberry fruit (*Morus alba* L.) with 1% FeCl3 solution. The results are shown by green, red, purple, dark blue, blue, blackish, or greenish-green.

Flavonoid test

The flavonoid test was performed by heating the ethanol extract of leaves and mulberry fruit for 5 min and then adds a few drops of the concentrated HCl and Mg powder. The results are indicated by the appearance of dark red.

Terpenoid

The terpenoid test is performed by reacting leaf and fruit extracts of mulberry (*M. alba* L.) with 0.5 mL of ethanol, 0.5 mL of anhydrous acetic acid, and 2 mL of concentrated sulfuric acid through the tube wall. Results are indicated by the formation of green and blue (triterpenoid) and red or purple (steroid) [49,67-93].

Data were analyzed descriptively qualitative. The data obtained are primary data from a phytochemical screening of methanol extract of bitter melon seed in the laboratory of organic chemistry and natural materials of Chemistry Department of Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

Immunohistochemistry of TGF-β of guinea pig testis

Testicular samples were taken from guinea pig by autopsy, then fixed with Bouin, end of fixation inserted in paraffin, and then cut with microtome with a thickness of 5 μm. Fixatives significantly improve the specificity and sensitivity of TGF-β while maintaining morphology in storage [84,94]. For obseravtion of histology of testis cell each guinea pig ± 10 field of view and calculated positive of TGF-β in seminiferous tubules with calculation criteria such as Table 1.

RESULTS

Based on the research that has been done in Medan, the results obtainedÐn various parameters ie:

Phytochemical testing of simplicia and seed extract of bitter melon seed

From data collection, the result of phytochemical testing of simplicia and bitter melon seed extract is presented in Table 2.

Table 1: The scoring system used

Score the proportion of staining	Intensity of nucleus staining	Added score
0=nucleus is not colored	0=no staining	0=no treatment response
1=<1% nucleus colored	1=weak staining	2-3=small treatment response (20%)
2=1-10% nucleus colored	2=moderate staining	4-6=medium treatment response (50%)
3=11-33% nucleus colored	3=strong staining	7-8=good response treatment (75%)
4=34-66% nucleus colored		
5=67-100% nucleus colored		

The maximum score of the addition is eight [40,94]

Table 2: Results of phytochemical testing of simplicia and bitter melon seed extract

Compound	Simplicia	Methanol extract
Phenolic	-	-
Flavonoid	+	+
Terpenoid	+	+

Scanning electron microscope (SEM)

SEM extract methanol seeds of bitter melon done in Indonesian science institutions (LIPI), Cibinong (Fig. 1).

DISCUSSIONS

The concentration of guinea pig spermatozoa after giving of bitter melon seed extract is shown in Fig. 2. The administration of the methanol extract of bitter melon seed until weeks 12 and 16 was significantly different ($p<0.05$) when compared between treatment and control. This suggests a good influence on suppressing spermatozoa concentrations produced by guinea pig of testicles. Spermatozoa concentration is highly determined on the process of spermatogenesis or stages of spermatozoa cell formation starting from spermatogonia, spermatocytes, and spermatids. Provision of bitter melon seed extract allows the decrease in testosterone levels in the testes (intratesticular testosterone) through repeated administration. Bitter melon seed extract contains β-Sitosterol with chemical structures similar to that of cholesterol. Cholesterol is a source of testosterone and enters the testicles so that the intratesticular testosterone is increased and consequently causes negative feedback to the hypothalamus and pituitary. The hypothalamus reduces the production of follicle-stimulating hormone-releasing hormone and luteinizing hormone-releasing hormone to affect the pituitary so that FHS and LH production is reduced [95-103]. The reduction of FSH affects Sertoli cells in producing ABP or testosterone receptors [104-110]. LH reduction suppresses Leydig cells to produce testosterone hormones [111-117]. These two ingredients are very important in the development and growth of spermatozoa cells so that production interruption will reduce the production of spermatozoa cells in the testes.

Beta-sitosterol D-glucoside is a phytosterol contained in *M. charantia* Linn. and several other plants [118-121]. Beta-sitosterol D-glucoside has many pharmacological activities [118-124] such as androgenic, antiadenomic, anticancer [129-134], antiedemic [135], and anti-inflammatory [136-142]. Sitosterol has a structure such as cholesterol that later in the body can become the precursor of testosterone and

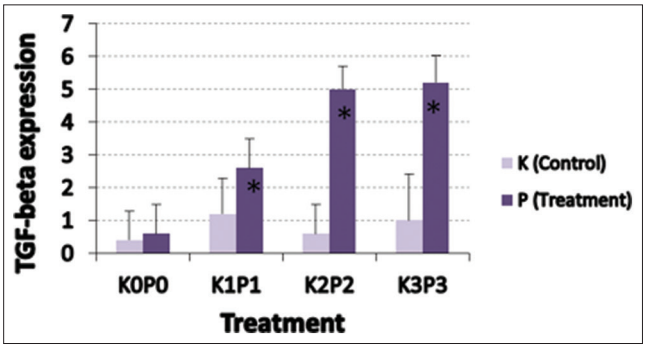

Fig. 1: Bar graphic of transforming growth factor beta of guinea pig. p*<0.05=the same pair between control and treatment, K0P0=control and treatment on week-0, K1P1=control and treatment on the week 4, K2P2=control and treatment on the week 8, K3P3=control and treatment on the week 12

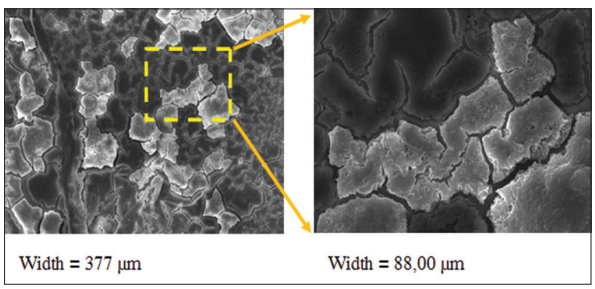

Fig. 2: Scanning electron microscope of extract methanol seeds of bitter lemon

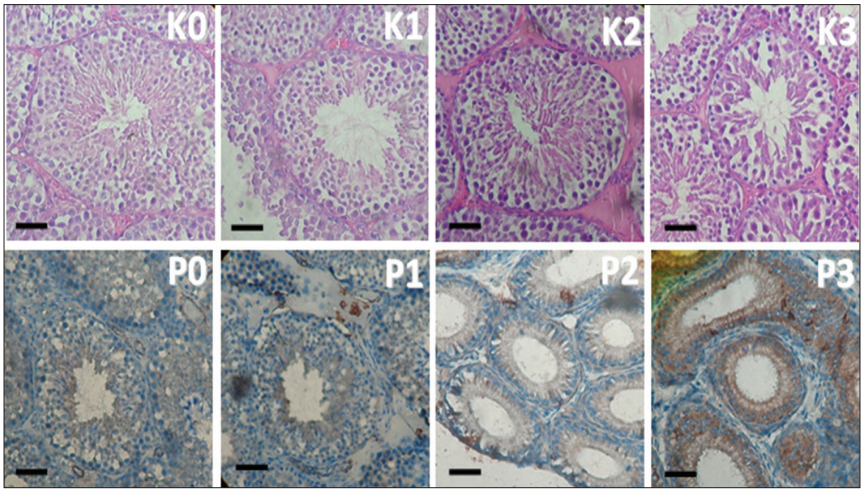

Fig. 3: Photomicrograph of seminiferous tubules in guinea pigs: K0P0= control and treatment on the week 0, K1P1=control and treatment on the week 4, K2P2=control and treatment on the week 8, K3P3=control and treatment on the week 12. K=HE;400×, P=IHC_TGF-β. Black line=200 µm

has been supposed to have an antifertility, and sex steroids have been implicated in the development and maintenance of Benign prostatic hyperplasia (BPH) and use of androgen reducing compounds, such as 5α-reductase inhibitors which block the conversion of testosterone into dihydrotestosterone [14,32,52]. So that if eating the extract of M. charantia seeds will cause increasedÐestosterone levels to the highest culmination point and will eventuallyÐead to decrease in testosterone of body serum, testes and disturb of TGF-β action in testis development [54,59,104]. Decreased testosterone in the body is replaced by DMPA. Hence, there is no decrease in libido [5,28,93].

Sitosterol contained in bitter melon seed (*M. charantia*) can activate transformation growth factor-β (TGF-β), causing apoptosis in spermatogenic cells and causing spermatozoa production to decrease and not even spermatozoa (azoospermia) (Fig. 3). That is, an increase in TGF-β causes a decrease in spermatogenesis so that spermatozoa are not formed. According to Carson and Rittmaster, TGF-β activity, which modulates apoptosis, is also influenced by dihydrotestosterone (DHT) [143,144]. More recently, the importance of DHT has been known to act as androgens and to be metabolized to 5α-androstane-3β-17β-diol (3βAdiol), androgens, which are a ligand for estrogen receptor [145-150].

CONCLUSIONS

Results showed that there was a significant effect ($p<0.05$) of methanol extract of bitter melon seed to increase the TGF-beta expression in the testis tubulus seminiferous. Therefore, it was able to be candidate for herbal contraception.

ACKNOWLEDGMENTS

The authors gratefully acknowledge that the present research is supported by the Ministry of Research and Technology and Higher Education Republic of Indonesia (Kemenristekdikti). The support is under the number of research grant: 003/SP2H/LT/DRPM/IV/2017, date – May 20, 2017 (the 2nd year of Competitive Grant).

AUTHORS' CONTRIBUTION

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors have declared that there are no conflicts of interest.

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