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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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 contraceptive options 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; dimethyl sulfoxide (DMSO) for 8 weeks; 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.

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 of 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 potant anti cancer agents, sopoletin 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 bitter 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.

Keywords: Testis histology, MDA, Bitter melon, Guinea pig.
**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*) 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 observation of histology of testis cell each guinea pig ± 10 field of view and calculated positive morphology in storage [84,94]. For observation of histology of testis cell each guinea pig ± 10 field of view and calculated positive morphology in storage [84,94]. For observation of histology of testis cell each guinea pig ± 10 field of view and calculated positive morphology in storage [84,94]. For observation of histology of testis cell each guinea pig ± 10 field of view and calculated positive morphology in storage [84,94]. For observation of histology of testis cell each guinea pig ± 10 field of view and calculated positive morphology in storage [84,94].

**RESULTS**

Based on the research that has been done in Medan, the results obtained on 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.

| 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 treatment (75%) |
| 4=34-66% nucleus colored        |                              |             |
| 5=67-100% nucleus colored       |                              |             |

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

**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 spermogenesis 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 testes 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 FSH 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 similar to that of cholesterol that later in the body can become the precursor of testosterone and

**Table 1: The scoring system used**

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

| Compound        | Simplicia | Methanol extract |
|-----------------|-----------|-----------------|
| Phenolic        | -         | -               |
| Flavonoid       | +         | +               |
| Terpenoid       | +         | +               |
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 increased/DiGesterone levels to the highest culminating point and will eventually dead to decrease in testosterone of body serum, testes and disturb of TGF-β action in testis development [5,9,104]. 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, 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 (3ΔAdiol), 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 tubules seminiferous. Therefore, it was able to be candidate for herbal contraception.

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AUTHORS’ CONTRIBUTION
All the authors have contributed equally.

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

REFERENCES
1. Ilyas S, Salomo H, Nurali A. Quantity and quality of guinea pig (Cavia porcellus) spermatozoa after administration of methanol extract of bitter melon (Momordica charantia) seed and depot medroxy progesterone acetate (DMPA) Quantity and quality of guinea pig (Cavia porcellus). IOP Conf. Series: Earth Environ Sci 2018;130:1-7.
2. DHS (Bangladesh). Bangladesh and Family Planning: An overview. Bangladesh: DHS; 2011. p. 1-8.
3. Ahmad N, Peng TN, Kamarul P, Kamarul F. Unfpa-icomp regional consultation. Fan Plan 2010; 2010:1-30.
4. Mansor M, Oo SS, Abdulla K. Prevalence of family planning practices among women influenced by husband’s socio demography and decision making. J Sains Kesihat Malay 2015;3:19-25.
5. Costantino A, Gava G, Berra M, Meriggiola Maria C. Advances in male hormonal contraception. Indian J Med Res 2014;140 Suppl: SS8-62.
6. Kanakis GA, Goulis DG. Male contraception: A clinically-oriented review. Hormones (Athens) 2015;14:598-614.
7. MacQuarrie KL., Edmeades J, Steinhaus M, Head SK. Men and Contraception: Trends in Attitudes and Use. DHS Analytical Studies; 2015.
8. Rashmi T, Jhuna S, Bhattacharya S. Bitter melon: A bitter body with a sweet soul. Int J Res Ayurveda Pharm 2011;2:443-7.
9. Uddin I, NS H. Bio-active compounds of bitter melon genotypes (Momordica charantia L.) in relation to their physiological functions. Funct Foods Heal Dis 2011;1:61-74.
10. Bakare RL, Magbagbeola O, Akinwande AI, Okunowo OW. Nutritional and chemical evaluation of Momordica charantia. J Med Plants Res 2010;4:2189-93.
11. Daniel P, Supe U, Royman MG. A review on phytochemical analysis of Momordica charantia. Int J Adv Pharm Biol Chem 2014;3:214-20.
12. Bakare RL, Magbagbeola OA, Akinwande AI, Okunowo OW, Green M. Antidiarrhoeal activity of aqueous leaf extract of Momordica charantia in rats. J Pharm Phyther 2011;3:1-7.
13. Mishra AK, Gautam A, Pal S, Mishra S, Rawat A, Maurya AK, et al. Innovate academic sciences effect of Momordica charantia fruits on streptozotocin-induced diabetes mellitus and its associated complications. Diabetic 2015;7:4-11.
14. Tumkiratiwong P, Ploypattarapanyo R, Pongchaierk U, Thong-Assa W. Reproductive toxicity of Momordica charantia ethanol seed extracts in male rats. Iran J Reprod Med 2014;12:695-704.
15. Tcheghebe OT, Timoléon M, Seukep AJ, Tatong FN. Ethnobotanical uses, phytochemical and pharmacological profiles, and cultural value of Momordica charantia Linn : An overview. Glob J Med Plant Res 2016;4:23-39.
16. Adewale OO, Oduyemi Ol, Ayokunle O. Oral administration of leaf extracts of Momordica charantia affect reproductive hormones of adult female wistar rats. Asian Pac J Trop Biomed 2014;4:5521-4.
Comparison of sexual dysfunction in women using depot-medroxyprogesterone acetate (DMPA-SC): A review. Int J Pharm Sci Res 2011:2:1135-46.

Beishuizen A, Thijss LG. Endotaxin and the hypothalamic-pituitary-adrenal (HPA) axis. J Endocrinol 2003;179:151-6.

Birkenhauer D, Battani MT. Hypothalamic and pituitary development: Novel insights into the aetiology. Eur J Endocrinol 2007;157:Suppl 1:S3-14.

Schmidt MV, Liebl C, Sterleman V, Ganea K, Hartmann J, Harbach D, et al. Neuroendocrine dysfunction in the hypothalamic-pituitary-adrenocortical axis. J Neuroendocrinology 2003;15:1056-7.

Keilberman D, Battani MT. Hypothalamic and pituitary development: Novel insights into the aetiology. Eur J Endocrinol 2007;157:Suppl 1:S3-14.

Reul JM. CRH neurons and stress: Novel insights into the pathophysiological consequences of the stress response. Neuroendocrinology 2001;73:115-20.

Ogbruwo PU, Unamba-Oparah IC, Odoemenam VU, Etuk IE, Okoli IC. The potentiality of medicinal plants as the source of new contraceptive agents. J Clin Diagn Res 2014;8:NC03-5.

Oliveira JS, Silva AA, Silva VA. Juvenile. Phytotherapy in reducing glycemic index and testicular oxidative stress resulting from induced diabetes: A review. Braz J Biol 2016;76:30-60.

Munell F, Suarez-Quian CA, Selva DM, Tirado OM, Reventos J. Androgen-binding protein and reproduction: Where do we stand? J Androl 2002;23:598-609.

Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: Past, present and future. Differentiation 2011;82:184-99.

Ma Y, Yang HZ, Xu LM, Huang YR, Dai HL, Kang XN, et al. Testosterone regulates the expression of androgen binding protein in rat sertoli cells. Sci Rep 2015;5:8994.

Morris ID, Bardin CW, Musto NA, Thau R, Gansalus GL. Androgen binding protein in serum, testis and epididymis following treatment with the leydig cell cytotoxic agent, ethylene dimethanesulphonate. J Androl 1990;11:1-153.

Ogbruwo PU, Unamba-Oparah IC, Odoemenam VU, Etuk IE, Okoli IC. The potentiality of medicinal plants as the source of new contraceptive agents. J Clin Diagn Res 2014;8:NC03-5.

Reul JM. CRH neurons and stress: Novel insights into the pathophysiological consequences of the stress response. Neuroendocrinology 2001;73:115-20.

Ogbruwo PU, Unamba-Oparah IC, Odoemenam VU, Etuk IE, Okoli IC. The potentiality of medicinal plants as the source of new contraceptive agents. J Clin Diagn Res 2014;8:NC03-5.

Oliveira JS, Silva AA, Silva VA. Juvenile. Phytotherapy in reducing glycemic index and testicular oxidative stress resulting from induced diabetes: A review. Braz J Biol 2016;76:30-60.

Munell F, Suarez-Quian CA, Selva DM, Tirado OM, Reventos J. Androgen-binding protein and reproduction: Where do we stand? J Androl 2002;23:598-609.

Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: Past, present and future. Differentiation 2011;82:184-99.

Ma Y, Yang HZ, Xu LM, Huang YR, Dai HL, Kang XN, et al. Testosterone regulates the expression of androgen binding protein in rat sertoli cells. Sci Rep 2015;5:8994.

Morris ID, Bardin CW, Musto NA, Thau R, Gansalus GL. Androgen binding protein in serum, testis and epididymis following treatment with the leydig cell cytotoxic agent, ethylene dimethanesulphonate. J Androl 1990;11:1-153.

Ogbruwo PU, Unamba-Oparah IC, Odoemenam VU, Etuk IE, Okoli IC. The potentiality of medicinal plants as the source of new contraceptive agents. J Clin Diagn Res 2014;8:NC03-5.
66. Henderson GI, Chen J, Schenker S. Ethanol, oxidative stress, reactive aldehydes, and the fetus. Front Biosci 1999;14:541-50.

67. Ad K, Mohrite R, Aggarwal AS, Ur S. Hepatoprotective medicinal plants of the cerebrum: a review. Asian J Pharm Clinical Res 2011;4:1-8.

68. Singh AB, Kaushal V, Megyesi JK, Shah SV, Kaushal GP. Cloning and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. Kidney Int 2002;62:106-15.

69. Imosemi O, Mitera LG, Novais PC, Tirapelli LF, et al. Apoptosis of Purkinje and granule cells: a stereological technique. Folia Morphol (Warsz) 2011;70:240-4.

70. Bregano LC. Expression of pro-and-anti-apoptotic antigens in the cerebellum of dogs naturally infected with canine distemper virus. Medica (B Aires) 2010;3:80-5.

71. Ghomari AM, Wehrle R, De Zeeuw CI, Sotelo C, Duraisami R, Mohite VA, Kasbe AJ. Anti-stress, adaptogenic activity of Zanthoxylum tinctorium. Asian J Pharm Clin Res 2010;3:1-3.

72. Paneerchelvan S, Lai HY, Kailasapathy K. Antioxidant, antibacterial and tyrosinase inhibiting activities of extracts from Myristica fragrans Houtt. Eur J Med Plants 2015;3:39-49.

73. Lossi L, Gambino G. Apoptosis of the cerebellar neurons. Histol Histopathol 2008;23:367-80.

74. Kotta LT, Cytotoxcicity effect of sea horse leaves. Asian J Pharm Clin Res 2011;4:92.

75. Satria D, Nasution MP, Illyas S. Cytotoxcicity effect of sea horse leaves. Asian J Pharm Clin Res 2011;4:92.

76. Harahap MU, Nasution MP, Ilyas S. The activity of kisspeptin-54 stimulates the hypothalamic-luteinizing hormone (LH)–gonadotropin–androgen (ADH): A working protocol. Int J Pharm Tech Res 2011;3:397-402.

77. Peng J, Wu Z, Wu Y, Hsu M, Stevenson FF, Boonplueang R, et al. Inhibition of caspase 3 protects cerebellar granule cells of the weaver mouse from apoptosis and improves behavioral phenotype. J Biol Chem 2002;277:44285-91.

78. Ghoumari AM, Wehrle R, De Zeeuw CI, Sotelo C, Dusart I. Inhibition and apoptosis induction of neuronal cell death. Neuron 2003;40:401-13.

79. Lotta LT, Cytotoxcicity effect of sea horse leaves. Asian J Pharm Clin Res 2011;4:92.

80. Yuan J, Lipinski M, Degterev A. Diversity in the mechanisms of neuronal cell death and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. Kidney Int 2002;62:106-15.

81. Harahap MU, Nasution MP, Ilyas S. The activity of kisspeptin-54 stimulates the hypothalamic-luteinizing hormone (LH)–gonadotropin–androgen (ADH): A working protocol. Int J Pharm Tech Res 2011;3:397-402.

82. Tai MC, Chen K, Cytotoxcicity effect of sea horse leaves. Asian J Pharm Clin Res 2011;4:92.

83. Oliveira SA, Chuffa LG, Fioruci-Fontanelli BA, Lizarte Neto FS, Badman MK, et al. Neuroprotection by taurine in ethanol-induced androgen- and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. Kidney Int 2002;62:106-15.

84. Yuan J, Lipinski M, Degterev A. Diversity in the mechanisms of neuronal cell death and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. Kidney Int 2002;62:106-15.

85. Tamaoka T, Kanato-Shinohara M, Lei Z, Rao CV, Murali A, et al. Apoptosis of Purkinje and granule cells of the cerebellum following chronic ethanol intake. Cerebellum 2014;13:728-38.

86. Adeyi AO, Jinadu AM, Arojoyoo AO, Alao OO, Ighodaro OM, Adeyi OE. In vivo and in vitro antibacterial activities of Momordica charantia on Salmonella typhi and its effect on liver function in typhoid-infected rats. J Pharmacogn Phyther 2011;5:183-8.

87. Akosman MS, Gocmen N, Kasapbas C, Karabekir HS. Estimation of purkinje cell quantification and volume in the cerebellum using a stereological technique. Folia Morphol (Warsz) 2011;70:240-4.

88. Bilan MS, Gocmen N, Kasapbas C, Karabekir HS. Estimation of purkinje cell quantification and volume in the cerebellum using a stereological technique. Folia Morphol (Warsz) 2011;70:240-4.

89. Harahap MU, Nasution MP, Ilyas S. The activity of kisspeptin-54 stimulates the hypothalamic-luteinizing hormone (LH)–gonadotropin–androgen (ADH): A working protocol. Int J Pharm Tech Res 2011;3:397-402.

90. Iliadou PK, Tsametis C, Kaprara A, Papadimas I, Goulis DG, et al. The role of antioxidants in cerebellar development. A review of literature. Int J Morphol 2013;31:203-10.

91. Beckett JL, Sakurai H, Adams BM, Adams TE. Moderate and severe nutrient restriction has divergent effects on gonadotroph function in orchidectomized sheep. Biol Reprod 1997;57:415-9.

92. Yu WH, Karanth S, Walczewska A, Sower SA, McCann SM. A hypothalamic follicle-stimulating hormone-releasing decapptide in the rat. Proc Natl Acad Sci U S A 1997;94:9499-503.

93. Schally AV, Kastin AJ, Arimura A. Hypothalamic follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-gonadotropin-releasing hormone: Structure, physiology, and clinical studies. Fertil Steril 1971;22:703-21.

94. Ahmadi R, Gohari A, Hooshmand M. The effect of noise stress on serum levels of LH, FSH and testosterone in male rats. Feyz 2015;19:24-29.

95. Calogero AE, Burrello N, Osino AM, Polosa P, D’Agata R. Activin-A stimulates hypothalamic gonadotropin-releasing hormone release by the explanted male rat hypothalamus: Interaction with inhibin and androgens. J Endocrinol 1998;156:269-74.

96. Dhilloo WS, Chaudhuri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, et al. Kisspeptin-54 stimulates the hypothalamic–pituitary–gonadal axis in human males. J Clin Endocrinol Metab 2005;90:6609-15.

97. Tehrani FR, Norozzadeh M, Zahediasl S, Piraye A, Azizi F. Introducing a rat model of prenatal androgen-induced polycystic ovary syndrome in adulthood. Exp Physiol 2014;99:792-801.

98. Rebourcet D, Ono-shaghnessy PJ, Monteiro A, Milne L, Cruickshanks L, Jeffrey N, et al. Sertoli cells maintain leydig cell number and peritubular myoid cell activity in the adult mouse testis. PLoS One 2014;9:e105893.

99. Rebourcet D, Darbeys, Monteiro A, Sofiintu E, Tsai YT, Handel I, et al. Sertoli cell number defines and predicts germ and leydig cell population sizes in the adult mouse testis. Endocrinology 2017;158:2955-69.

100. Tanaka T, Kanato-shinohara M, Lei Z, Rao CV, Shainohara T. The luteinizing hormone-testosterone pathway regulates mouse spermotogenic step cell self-renewal by suppressing Wnt5a expression in sertoli cells. Stem Cell Reports 2016;7:729-91.

101. Allan CM, Garcia A, Spaliviero J, Zhang FP, Jimenez M, Huhtaniemi I, et al. Complete sertoli cell proliferation induced by follicle-stimulating hormone (FSH) independently of luteinizing hormone activity: Evidence from genetic models of isolated FSH action. Endocrinology 2004;145:1587-93.

102. Abdi MH, Baker PJ, Charlton HR, Monteiro A, Verhoeven G, De Gendt K, et al. Spermato genesis and sertoli cell activity in male mice lacking sertoli cell receptors for follicle-stimulating hormone and androgen. Endocrinology 2008;149:3279-85.

103. Walker WH, Cheng J. FSH and testosterone signaling in sertoli cells. Reproduction 2005;130:15-28.

104. Hidou PK, Tsimetis C, Kaprara A, Papadimas I, Goulis DG. The sertoli cell: Novel clinical potentiality. Hormones (Athens) 2015;14:504-14.

105. Ilkhizanideh B, Taghizadeh M, Mahzad-sadaghian M. Bilateral leydig cell tumor and male infertility: A case report. Iran J Reprod Med 2015;13:47-9.

106. Setchell BP, Pakarinen P, Huhtaniemi I. How much LH do the leydig cells see? J Endocrinol 2002;175:375-82.

107. Obiorah IE, Kyrillos A, Ozdemirli M. Case report synchronous leydig cell tumor and seminoma in the ipsilateral testis. Case Reports Urol 2015;4:e105893.
spematogenesis in. Zool Stud 2010;49:39-50.
116. Hibi H, Yamashita K, Sumitomo M, Asada Y. Leydig cell tumor of the testis, presenting with azoospermia. Reprod Med Biol 2017;16:392-5.
117. Opalka M, Kaminiska B, Ciereuzko R, Dusza L. Genistein affects testosterone secretion by leydig cells in roosters (Gallus gallus domesticus). Reprod Res 2004;1:845-93.
118. To K, Oe Y, Ao O. Effect of Carica papaya bark extract on oxidative stress parameter tests in male albino rats. Int J Appl Res Nat Prod 2012;4:1-6.
119. Okoro VM, Mbajorgu CA, Mbajorgu EF. Semen quality characteristics of koekoek breeder cocks influenced by supplemental inclusion levels of onion and garlic mixture at 35-41 weeks of age. Rev Bras Zootec 2016;45:433-40.
120. Yama OE, Duru FI, Oremosu AA, Noronha CC, Okenlawon A. Suppressive effects of Momordica charantia on pituitary-testicular axis and sperm production in male Sprague-Dawley rats. Int J Med Sci 2011;3:353-9.
121. Eboetse YO, Ikechukwu DF, Olugbenga OA, Ayodele OA, Caramel NC. The influence of extraction solvents on the anticancer activities of Tinospora cordifolia and Barleria grandiflora. J Pharm Sci 2013;5 Suppl.1:245-9.
122. Eilam D, Golani I. Home base behavior in amphetamine-treated tame wild rats (Rattus norvegicus). Iran J Med Sci 1990;36:161-70.
123. Setiawan A, Sagi M, Asmara W. Analisis kuantitatif sel purkinje dalam kerutan cerebellum mencit (Mus musculus) setelah induksi ochratoxin a selama periode organogenesis quantitative analysis of the purkinje cell in mice cerebellum after induction of ochratoxin a during organogenesis period. J Biol Papua 2011;16:3.
124. Ju YH, Clausen LM, Allred KF, Alnada AL, Helferich WG. Beta-sitosterol, beta-sitosterol glucoside, and a mixture of beta-sitosterol and beta-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells in vitro and in ovariectomized athymic mice. J Nutr 2004;134:1145-51.
125. Arora M, Kalia AN, Mishra R, Siddqui AA. Isolation and characterization of stigmasterol and β-sitosterol glucoside from the extract of the flowers of Ilyas et al. Hirenath MB, Hiremath MB et al. Pharmacognosy and phytochemistry of the leaves of Ipomoea digitata (Agrimonia pilosa) extract: Comparison of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of Ipomoea digitata. J Immunotoxicol 2016;13:127-35.
126. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA. Evaluation of the anti-inflammatory activity of the aqueous and ethanol extracts of the leaves of Albizia lebbeck in rats. J Tradit Complement Med 2016;6:172-5.
127. Saeidnia S. The story of beta-sitosterol - a review. Eur J Med Plants 2014;4:590-609.
128. Hossain MS, Khan NM. Scopoletin and β-sitosterol glucoside from the flowers of Viola odorata. Br J Pharm Res 2017;16:1-8.
129. Saednia S. The story of beta-sitosterol- a review. Eur J Med Plants 2014;4:590-609.
130. Hossein MS, Khan NM. Scopoletin and β -sitosterol glucoside from roots of Ipomoea digitata. J Pharm Phytochem 2015;5:3-7.
131. Prakash O, Kumar A, Kumar P, Ajeez A. Antiacancer potential of plants and natural products: A review. Am J Pharm Res 2013;3:1-10.
132. Jawad A, Balayethsshwath RV, Rami A, Waleed R, Hatem S, Nathan WL. The influence of extraction solvents on the anticancer activities of Palestinian medicinal plants. J Med Plants Res 2014;8:408-15.
133. Manglani N, Vaishnava S, Dhanmodar P, Sowarkar H. In vitro and in vivo anti-cancer activity of leaf extract of Barleria grandiflora. Int J Pharm Phytochem 2014;6:14-6.
134. Ghagane SC, Puranik SI, Kumar VM, Nerli RB, Jalalpure SS, Hirmath MB, et al. In vitro antioxidant and antiacancer activity of Leva indica leaf extracts on human prostate cancer cell lines. Integre Med Res 2017;6:79-87.
135. Solowey E, Lichtenstein M, Ballon S, Paavilainen H, Solowey E, Lorberboum-Galinski H. Evaluating medicinal plants for anticancer activity. Sci World J 2014;2014:12.
136. Arianto A. Antilucler effect of gastroretentive spherical matrices of alginic-chitosan containing ranitidine HCL. Int J Pharm Tech Res 2016;9:342-52.
137. Ashour AA, Raafat D, El-Gowelli HM, El-Kamel AH. Green synthesis of silver nanoparticles using cranberry powder aqueous extract: Characterization and antimicrobial properties. Int J Nanomedicine 2015;10:7207-21.
138. Saktiveel KM, Guruvayoorappan C. Acacia ferrariginea inhibits inflammation by regulating inflammatory INOS and COX-2. J Immunotoxicol 2016;13:127-35.
139. Setiawan A, Sagi M, Asmara W. Analisis kuantitatif sel purkinje dalam kerutan cerebellum mencit (Mus musculus) setelah induksi ochratoxin a selama periode organogenesis quantitative analysis of the purkinje cell in mice cerebellum after induction of ochratoxin a during organogenesis period. J Biol Papua 2011;16:3.
140. Leea indica. J Pharm Phytochem 2015;4:5-7.
141. Momordica charantia. J Pharm Phytochem 2015;4:5-7.
142. de Oliveira RG, Mahon CP, Ascêncio PG, Ascêncio SD, Balogun SO, de Oliveira Martins DT, et al. Evaluation of anti-inflammatory activity of hydroethanolic extract of dilodendron bipinnatum radlk. J Ethnopharmacol 2014;155:387-95.
143. Weng Y, Xue F, Xu L, Zagorevski D, Spink DC, Ding X, et al. Analysis of testosterone and dihydrotestosterone in mouse tissues by liquid chromatography-electrospray ionization-tandem mass spectrometry. Anal Biochem 2010;402:121-8.
144. Lee PW, Swerdloff RS. NIH public access. Biosystems 2009;73:1345-52.
145. Seidlova-Wuttke D, Hesse O, Jarry H, Christoffel V, Spengler B, Becker T, et al. Evidence for selective estrogen receptor modulator activity in a black cohosh (Cimicifuga racemosa) extract: Comparison with estradiol-17beta. Eur J Endocrinol 2003;149:351-62.
146. Lee YM, Kim JB, Bae JH, Lee JS, Kim PS, Jang HH, et al. Estrogen-like activity of aqueous extract from Agrimonia pilosa lebed. in MCF-7 cells. BMC Complement Altern Med 2012;12:260.
147. Miyuki K, Nobuhiro S, Daimei S, Hidehiko S, Yoshimichi M, Kunimasa A, et al. Sex differences in the serum level of endogenous 7 cells. BMC Complement Altern Med 2012;12:260.
148. Lee YM, Kim JB, Bae JH, Lee JS, Kim PS, Jang HH, et al. Estrogen-like activity of aqueous extract from Agrimonia pilosa lebed. in MCF-7 cells. BMC Complement Altern Med 2012;12:260.
149. Joshia SC, Sharma A, Chaturvedib A. Antifertility potential of some medicinal plants in males: An overview. Int J Pharm Pharm Sci 2011;3:204-17.
150. Pakhira BP, Jana K, Ghosh A, Ghosh D. Antitesticular activity of hydro-methanol extract of dilodendron bipinnatum radlk. J Immunotoxicol 2016;13:127-35.