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Submission date: 07-Jan-2021 10:38AM (UTC+0700)
Submission ID: 1483949464
File name: Arjadi_2019_IOP_Conf._Ser.__Earth_Environ._Sci._255_012022.pdf (1.44M)
Word count: 3540
Character count: 19039

IOP Conference Series: Earth and Environmental Science

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To cite this article: F Arjadi *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **255** 012022

PURWOCENG ROOTS ETHANOL EXTRACT MAKE NO IMPROVEMENT IN LEYDIG CELLS ACTIVITY TO MALE WHITE RATS (*Rattus norvegicus*) EXPOSED BY PARADOXICAL SLEEP DEPRIVATION (PSD) STRESS MODELS

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Abstract. To observe the improvement of Leydig cell activity after the administrations of Purwoceng roots ethanol extract white male rats which is exposed by PSD stress model**.** Experimental research, pre and post with control group study design**.** White male rats (*Rattus norvegicus*) wistar strain animals were divided into six groups, five rats each group, there are negative control group (KI), PSD control group without sleep recovery (KII), PSD control group with sleep recovery (KIII), and also PSD group with Purwoceng roots ethanol extract with the dose of 16,65-16,75 mg, 33,30-33,75 mg and also 50,25 mg/200 gramBW/day (KIV, KV, KVI). PSD stress model exposure was administrated in 96 hours, continued by sleep recovery and intervention method in the next seven days after it and then leydig cell number and serum testosterone level r is examined. Statistical analysis shown that in leydig cell number study (p value = 0,589) and serum testosterone level study (p value = 0,572) so the result is not significant**.** Administration of Purwoceng roots ethanol extract make no significant effect in leydig cell number and serum testosterone level (p>0,05) so there is no improvement of Leydig cell activity in white male rats which is inducted by PSD stress model.

Keyword: Leydig cell activity, purwoceng, paradoxical sleep deprivation (PSD)

1. Introduction

Sleep is basic needs in human life. Average normal sleep duration average is 8 hours/day with the tolerance sleep duration is 6 hours/day to support work performance, but every person has their own sleep duration needs, depend on the circadian rhythm. Some people only have 3-5 hours/day duration of sleep. If someone doesn't get optimal sleep duration or even no sleep at all, they will get physiological disturbance and impairment.[1] Paradoxical sleep (PS) is one of sleeping phase with intense brain activity same as when people is awaken Another term of PS is Rapid Eye Movement (REM) phase. REM phase is placed in the beginning phase of sleep duration, characterized by dreaming and weakening of motoric function except eye and diaphragm muscle. Sleep deprivation (SD) is the suppression of sleep duration which can make human lost some or even all of their sleep duration. This condition is Paradoxical Sleep Deprivation (PSD).[2]

Stress exposure to experimental animals or to human by every model can increase the activity of hypothalamus-pituitary-adrenal (HPA) axis. Stress can induce corticotrophin releasing hormone (CRH) release and then CRH can make pituitary gland release adrenocorticotrophic hormone (ACTH) and then ACTH can make adrenal gland release stress hormone, glucocorticoid.[4] Glucocorticoid have important role in reproduction quality suppression by several mechanism, such as: 1) Glucocorticoid can suppress hypothalamus-pituitary-testis (HPT) axis so suppression of Gonadotrophic releasing hormone (GnRH) by hypothalamus and also Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) in pituitary gland and testosterone by testis. This suppression finally can also suppress testosterone level and leydig cell number because its proliferation is depend on testosterone effect.[5] 3) Glucocorticoid can suppress the production of beta-hydroxisteroid dehidrogenase-1 (11βHSD1) which

have role in protecting steroidogenesis process in testis from the bad effect of glucocorticoid.[6] 4) Glucocorticoid can disrupt metabolism which can induce release of free radicals compound such as Reactive oxygen species (ROS) which can induce apoptosis of reproduction cell, such as leydig cell. [7]

Purwoceng (*Pimpinella alpina Molk/ Pimpinella pruatjan Molk*) is one of traditional herbal medicine which is original from Indonesia and have some good effect to reproduction function. All part of the plant (roots, trunk, and leafs) can be used as reproduction stimulator agent and also androgenic agent.[10,11] Purwoceng roots also has many active substance many active substace, in example, flavonoid and tannin is exogen antioxidant which can increase antioxidant level in the body and suppress the formation of free radicals such as ROS so testis tissue damage can be prevented so it can heal the sertoli cell number, spermatogenic score, leydig cell apoptosis and also decrease testis MDA level so the reproduction function will be elevated.[12,13] Euricomalacton and amarolinda are androgen compound that can increase the sensitivity of GnRH receptor in hypophysis so it can increase the release of FSH and LH and finally can increase testosterone level and leydig cell number and reproduction function will be healed.[1]

2. Materials and Methods

The research is using true experimental pre and posttest with control group study design. This research is done in two variables of Leydig cell activity, there are leydig cell number and serum testosterone level. Experimental study used in this research is 30 white male rat (*Rattus norvegicus*) Wistar strain aged 2-3 months with 150-250 gram weight. The experimental animals were divided into 6 groups of treatment, each contains 5 rats with weight of 150-250 gram. Group I is negative control group, it is group that is not exposed by PSD stress model but not administrated by Purwoceng root ethanol extract. Another five group is exposed by PSD stress model using modified multiple platform method (MMPM). Group II is PSD control group which is not continued by sleep recovery but continued by termination with decapitation method. Group III is PSD control group which is continued by *sleep recovery*. Group IV, V, and VI is group which is exposed by PSD stress model and continued by various dose of Purwoceng root ethanol extract which is given differently to every group. But, In serum testosterone study, there is are only five group of rats, the difference is KII and KIII is merged and not separated by sleep recovery into positive control group (II), with the remaining group is still exist with different number but same order, KI is negative control group, KIII, KIV and KIV is same as the KIV, KV and KVI group in the study other than serum testosterone level.

PSD treatment is applied by placing rats in a 123 x 44 x 44 cm cage to apply MMPM method. MMPM cage is featured by 18 platforms with distance each platform is 10 cm so the rats can move freely and interact with another rats. The base of cage contain 1 cm-height water. PSD stress is exposed by automatically turn on muscle atonia tools which can make sleeping rats fallen into the water and be awaken. Before treatment, acclimatization is done in 7 days. PSD is exposed in 96 hours (4 days) after acclimatization and continued by treatment another 7 days. The difference in each group can be seen in table below :

Table 1. Rats Treatment Timeline

Group	Days 1-7	Days 8-11	Days 12-18	Days 19
Negative control	Acclimatization	Without PSD	Aquadest	Termination
PSD with *sleep recovery*	Acclimatization	PSD	Aquadest	Termination
PSD without *sleep recovery*	Acclimatization	PSD	Termination	
PSD + Purwoceng 16,75 mg/200 gBW/day	Acclimatization	PSD	Purwoceng 16,75 mg/200 gBW/day	Termination
PSD + Purwoceng 33,75 mg/200 gBW/day	Acclimatization	PSD	Purwoceng 33,75 mg/200 gBW/day	Termination
PSD + Purwoceng 50,25 mg/200 gBW/day	Acclimatization	PSD	Purwoceng 50,25 mg/200 gBW/day	Termination

The data collection of the Leydig cell was from histopatologic examination and testosterone serum level from ELISA assay and analysis with Kruskall-Wallis. All experimental procedures were approved by the Medical Research Ethics Committee, Faculty of Medicine, Jenderal Soedirman no.016/KEPK/III/2014.

3. Results and Discussion

The results of the research was shown in the tables and picture below :

Table 2. Observation Result of Purwoceng Root Ethanol Extract on Group I-VI with Various Type of Treatment

Group / Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	P value
Leydig cell number	11,96±3,81	8,94±2,94	10,70±2,70	9,62±0,71	10,12±3,25	10,82±1,36	0,589
Serum Testosterone Level	2,61±2,38	1,52±0,76		1,45±0,70	1,99±1,53	0,97±0,33	0,572

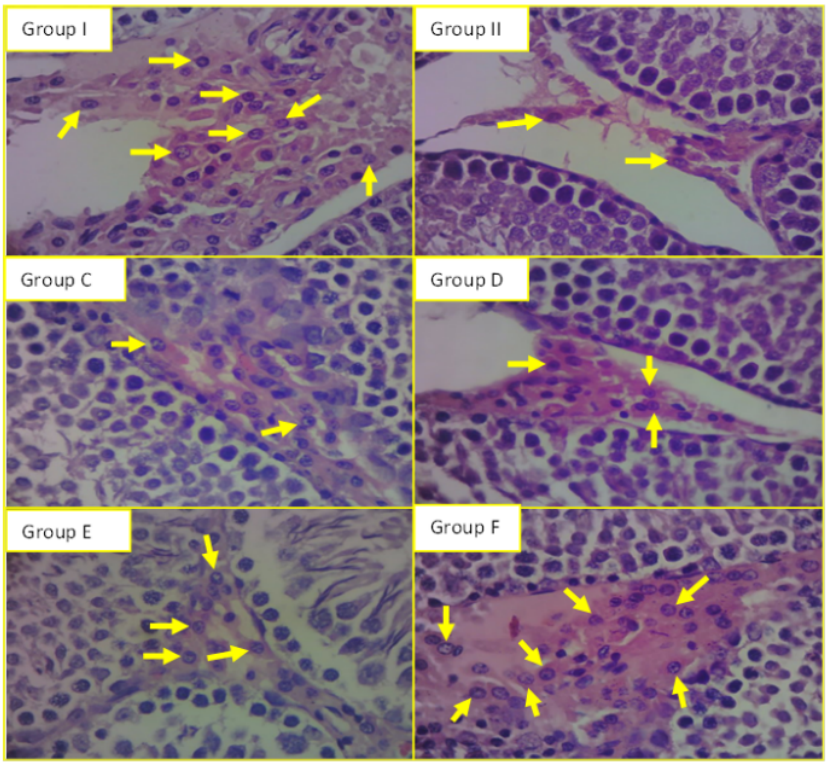

Figure 1. Histological view of the number Leydig cells of white rat *Rattus norvegicus testicle (HE, 400 X)* in each intervention (Leydig cells : round nucleus, big, in the center of cell, eosinofilic cytoplasm (yellow arrow)

3.1. Leydig cell number

Stres exposed rats could increased corticosteroid hormone and adrenalin, that caused rises of oxidative stress and decreasing androgen hormone level and *messenger Ribonucleic Acid* (mRNA) trancription that caused the failure of the differentiation of Leydig cells. [14]. Rat that induced with PSD stress models experienced the increasing of oxidative stress that shown with the accumulated of peroxidation lipid and decreasing of endogen antioxidants (glutathion).[15] The accumulated of reactive oxidant species (ROS) could impact the breaking of cell membrane structure that consisted of *lipid bilayer* with peroxidation lipid (the enzyme that took electron from cell membrane lipid).[16] Lipid peroxidation could impact with the disturbance of essential ion transport in and out of cell that could make the breaking and death of Leydig cell that decreasing the number of Leydic cell.[17] Reactive oxidant species could broke the *mitochondrial deoxyribonucleic acid* (mtDNA) and speed up the aging process of Leydig cells that shown with the rise of cellular lipofuchsin.[18]

There is a difference between leydig cell numbers in groups I and III. Group I has a higher average number of Leydig cells than group III (PSD without sleep recovery). This happens because the PSD treatment can cause stress reaction in animals that affect the Leydig cells. Stress experienced by animal impacting on the raising of glucocorticoid resulting in abnormal apoptosis and impaired Leydig cell differentiation due to inhibition of GnRH.[19] Difference in the average Leydig cells number between groups I and III group were not statistically significant ($p > 0.05$). Results were not significant because PSD exposure duration for 96 hours is not adequate enough to damage the Leydig cell. The suppression in the number of Leydig cells can occur significantly after stress model of PSD for 25 days. Leydig cell count decline significantly also can occur after exposure to the stress model of PSD for 7 days.[20]

The differences in the average number of Leydig cells also occur in groups II and III. Group II has lower result than the group III. Sleep recovery time is expected to increase endogenous antioxidants which can have a protective effect against apoptosis, a suppression in the concentration of glucocorticoids and even increase the levels of LH. There is no improvement in levels of testosterone but in the other side we can find suppression in sleep recovery group after 5 days. Sleep recovery time also did not produce a significant increase in the levels of LH because age affects the ability of the tissue to recovery. Recovery failure in experimental animals is occurred because the process of sounding given daily the animals give extra stress effect.[2]

In group IV, V, VI there is escalation in the average number of Leydig cells is proportional to the number of Purwoceng extract given. Furthermore, in group VI we have increased mean results that are almost as same as the group I. In this study, no significant results obtained from each Purwoceng extract dose given due to the dose purwoceng extract given is not adequate enough to be able to affect the number of Leydig cells significantly. The is no report that the dose has effect on Leydig cells.[21]

3.2. Serum testosterone levels

Normal levels of testosterone rats is 0.66 to 5.4 ng/mL and statistical test results in this study shows significant mean variation of serum testosterone levels in five groups. Group I has mean testosterone levels were highest among other groups and it is higher than normal. The mean serum testosterone levels in the group given PSD stress is lower compared to a healthy control group. Group II experienced a mean decrease in testosterone levels. The mean serum testosterone levels in group II is lower than normal value of testosterone and stress than in group I. PSD will result in mice undergoing physical and psychological stress due to loss of sleep time.[2]

Rats after exposure the stress model of PSD for 3 days compared with the control group shown a significant decreasing of serum testosterone concentration.[2] LH concentrations decreased significantly compared to the controls on the fifth day post-exposure model of stress PSD. After PSD stress models exposure for 96 hours, 42 rats were given a 96-hour recovery sleep does not cause testosterone levels back to normal.[5] Twenty-four hours after exposure to the PSD, testosterone levels decreased by 57% compared to the controls and the levels will continue to decline up to 96 hours of PSD. Post-sleep recovery testosterone levels begin to rise after 24 hours, but the provision of recovery sleep for 96 hours has not been able to make such levels of testosterone back to normal.[2,5]

The group presented a model of PSD stress and continued with the purwoceng extract showed various average testosterone levels. Group III experienced a mean decrease in testosterone levels but

the number is almost like group II. This is presumably due to the dose of the purwoceng extract inadequate in increasing testosterone. Research previously stated that at a dose of 25 mg / 300 gBW / day, equivalent to 16.65 mg / 200 gBW / day has not been able to induce pharmacological response.[22] This situation is caused by the low concentration of purwoceng extract which is circulating in the blood. The low concentration is not effective to hold coupling with cell receptors to evoke change and pharmacologic response.

Group IV had a higher mean testosterone levels than the control group pain and the other two groups were given a root extract purwoceng. This is presumably because the purwoceng extract dose approaching the optimal dose. These results are consistent with Taufiqurahman (2009) through similar research, that there is a significant increase in mean testosterone levels of white rats treated for 7 days with 43 doses of 2 ml (50 mg) purwoceng extract (mean level of 2.31 ± 1 , 52) compared with controls (mean score = 1.00 ± 0.31; p = 0.047).[23] Group V has the lowest average value of testosterone levels among other groups. The emergence of pharmacological drug response is determined by the concentration of drug in circulation, the number of receptors and receptor in the binding affinity of the drug. The third determining factor is not to be further investigated regarding its role in the increase in the dose which results in decreased levels of testosterone. Until now there has been no in-depth research on purwoceng dose either in the form of botanicals, extracts and herbal.[22] In this study, no significant results obtained from each dose given purwoceng extract. This happens because the purwoceng extract is not given adequately enough to be able to affect the significant increase in testosterone levels.

4. Conclusion

Administration of Purwoceng roots make no significant positive effect of Leydig cell activity after exposed Paradoxical Sleep Deprivation in white male rats

Acknowledgements

The authors thank the Directorate General of Higher Education, Ministry of Education and Culture, for research funding through the Hibah Fundamental scheme and thanks are also due to the head of the Pathology Anatomy Laboratory and Experimental Animal Laboratory, Medical Faculty, Jenderal Soedirman University for the using of their research facilities

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