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Oral Administration of *Cassia Alata* L. Leaves Ethanol Extract Prevented the Decrease of Leydig Cell Number but Did Not Decrease of Testosterone Level in Male Wistar Rats (*Rattus Norvegicus*) Exposed to Cell Phone Radiation

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Abstract: ***Background*: In the modern world today, humans are often exposed to electromagnetic waves, especially those originating from cell phones. Cell phone electromagnetic radiation can trigger stress, thus causing the decrease of Leydig cells number and also the decrease of testosterone levels. Cassia alata L. leaves ethanol extract contains bioactive compounds such as flavonoids, phenols, tannins, and antioxidants. The purpose of this study was to prove oral administration of Cassia alataL. leaves ethanol extract prevents the decrease at Leydig cell number and the decrease of testosterone levelin male Wistar rats (Rattus norvegicus) exposed to cell phone radiation. *Methods*: This study used a randomized posttest only control group design. Subjects were 36 white rats, male, Wistar strain, healthy, 6 months old, weight180-200gram, that were divided into two groups (18 rats/group). The control group was given a placebo (2 ml aquabidest) and exposed to cell phone radiation, the treatment group was given ethanol extract of Cassia alata L. leaves 2 mg/200 gr BB diluted in 2 ml aquabidest and exposed to a cell phone for 2 hours every day in 21 days. After treatment, the number of Leydig cells was examined histopathologically with HE staining and testosterone levels were examined by the ELISA method. *Results*: The results showed that the median number of Leydig cells in the control group was 12.7 (10.8-23.6) cells/field of view significantly lower compared with treatment group 27.9 (9.80-35.8) cells/field of view (p<0.001). Testosterone levels in the control group 28.8 (14.7-37.1) nmol / L, did not significant difference with treatment group31.5 (16.8-38.4) nmol / L (p = 0.103). *Conclusion*: It can be concluded that oral administration of Cassia alataL. leaves ethanol extract prevented the decrease ofLeydig cell number but did not decreaseoftestosterone levelin male Wistar rats (Rattus norvegicus) exposed to cell phone radiation.***

Keywords: *Cassia alata*L.Leaves ethanol extract, Leydig cells, testosterone, cell phone radiation

1. Introduction

Aging is a natural process that will be experienced by every living thing. It is caused by two groups of factors, namely internal factors (free radicals, hormonal deprivation, glycosylation processes, apoptotic, methylation, immune system impairment and genes), and external factors (unhealthy lifestyle and habits, environmental pollution, poverty and stress).[1] One indicator of the aging process is the decrease in testosterone,[1,2], therefore, prevention of decreasing testosterone caused by cell phone radiation can be used as an Anti Aging Medicine (AAM) step. Currently, a lot of research is done to find any natural ingredients that contain antioxidants to inhibit the activation of the Hypothalamus-Pituitary-Adrenal (HPA) axis which can prevent a decrease in testosterone levels so that the aging process can be inhibited.

The total flavonoid content in several plants in Waitina Village, East Mangoli District, Sula Islands Regency, North Maluku, obtained results that showed that the highest total flavonoid content in *Cassia alata* L. plants was 26.8633mg/mL.[3]

The hypothalamus is the master regulator of most systems in the body including the stress response axis. Stressful conditions due to exposure to electromagnetic waves will trigger excess production of cortisol via the HPA axis.[4] The response initiates by stimulation of the hypothalamus, increasing the production of Corticotropin-Releasing Hormone (CRH) which further stimulates adrenocorticotropic hormone (ACTH) secretion from the pituitary. ACTH activates cortisol production in cortex adrenal and functions as anti-inflammatory and immunosuppressive.[4-7] Cortisol also binds to its receptors in the hypothalamus, inhibiting the secretion of GnRH, thus decreased LH and FSH secretion by the pituitary. As a result of decreased LH secretion, the activity of steroidogenesis by Leydig cells in producing testosterone will decrease.[8]

The hypothesis of this study was *Cassia alata* L. leaves ethanol extracts can stimulate GnRH secretion from the hypothalamus, increased LH and FSH secretion from the pituitary, then affected Leydig cells to secrete testosterone, it was expected that the flavonoid content of *Cassia alata* L. leaves ethanol extracts can prevent the decline of Leydig cells and also testosterone due to cell phone radiation-induced oxidative stress. Increased activation of Leydig cells and testosterone secretion is expected to prevent and inhibit the aging process and overcome reproductive disorders. However, empirical proof through experimental research is required. Therefore, the aim of this study was to prove oral

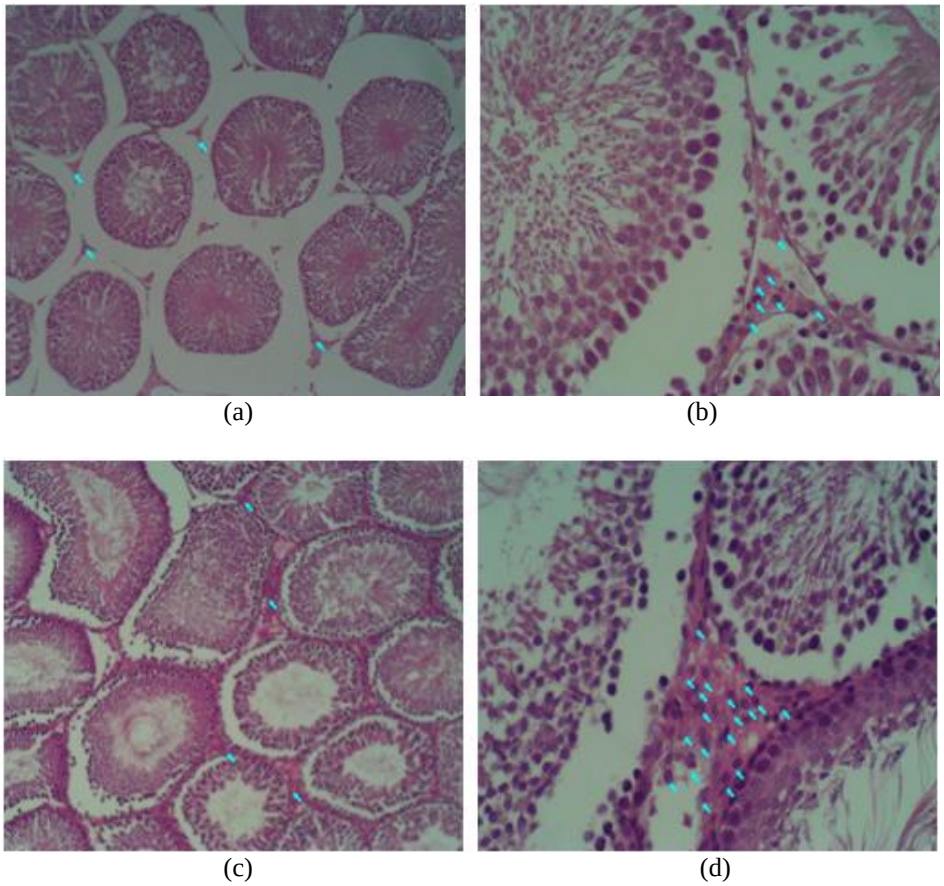
administration of *Cassia alata*L. leaves ethanol extract prevented the decrease in Leydig cell number and the decrease of testosterone level in male Wistar rats (*Rattus norvegicus*) exposed to cell phone radiation.

2. Methods

This study used a randomized posttest only control group design. Subjects were 36 white rats, male, Wistar strain, healthy, 6 months old, weight180-200gram, that were divided into two groups (18 rats/group). The control group was given a placebo (2 ml aquabidest) and exposed to cell phone radiation, the treatment group was given ethanol extract of *Cassia alata* L. leaves 2 mg/200 gr BB diluted in 2 ml aquabidest and exposed to a cell phone for 2 hours every day for 21 days. After treatment, the number of Leydig cells was examined histopathologically with HE staining and testosterone levels were examined by the ELISA method.

3. Results

The comparative analysis using showed that the median number of Leydig cells in the control group [12.7(10.8-23.6) cells/ field of view was significantly lower compared with the treatment group 27.9(9.80-35.8) cells/field of view; $p<0.001$]. But, the testosterone levels in the control group [28.8(14.7-37.1) significant defference withthe treatment group 31.5(16.8-38.4) nmol/L; $p = 0.103$).



(a) (b) (c) (d)

Figure 1: Histopathology of Leydig Cell collected from Control Groups (a, b) and Treatment Groups (c, d) (blue arrows indicate Leydig cells; with100x and 400x magnification)

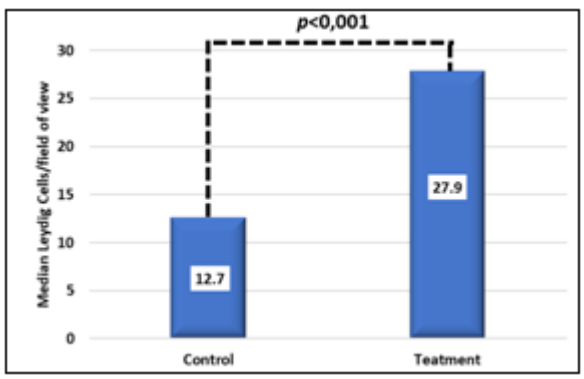


Figure 2: Median Leydig Cell Numbers Comparison

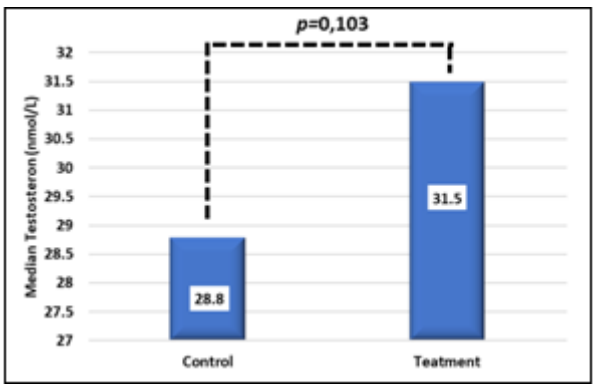


Figure 3: Median Testosteron Levels Comparison

4. Discussion

The effect of *Cassia alata*L. Leaves Ethanol Extracton Leydig Cells

To prevent a decrease in the Leydig cells number due to exposure to cell phone radiation, we used oral administration of *Cassia alata* L. leaves ethanol extracts. This is because the *Cassia alata* L. leaves ethanol extracts to contain several bioactive compounds that have been proven in many previous studies to prevent degenerative damage to cells, which in this study focused on Leydig cells. We found that *Cassia alata* L. leaves ethanol extracts used in this study contained phenols (2975.10 mg/100g GAE), flavonoids (1128.79 mg/100g), tannins (4176.05 mg/100g TAE), and antioxidants total (3465.65 mg/L) with IC50 of 372.8930 ppm. It is supported by the previous study showed the total flavonoids in *Cassia alata* L. Plants was 26.8633mg/mL.[3] Examination of Leydig cell numbers showed that the treatment group with *Cassia alata* L. leaves ethanol extracts had a higher number of Leydig cells compared to the control group with placebo. These results show the positive effect of *Cassia alata* L. leaves ethanol extracts in preventing Leydig cell depletion in male Wistar rats exposed to cell phone radiation. This effect is most likely due to the bioactive compound in the *Cassia alata* L. leaves ethanol extracts and not the antioxidant content. Antioxidants are stable compounds to donate free electrons and neutralize them, thereby reducing the capacity of free radical damage.[9]

Exposure to cell phone radiation can cause elevated cortisol levels.[4] This cortisol then inhibits the activity of Leydig cells because cortisol can inhibit the secretion of GnRH, stimulating the pituitary to secrete LH and FSH.[8] Phenols and flavonoids have been shown to actively ameliorate stress responses.[10] Flavonoids can increase the response of GR receptors activity and reduce levels of CRF and ACTH thereby preventing an increase in cortisol.[11] Flavonoids can also prevent the activation of the HPA axis including inhibition of stress hormone levels and upregulation of the hippocampal GR and also has been proven to prevent damage to PC12 nerve cells caused by high corticosterone due to its binding to 5-HT1A.[12]

Moreover, flavonoids significantly reduce plasma corticosterone and adrenocorticotropic hormone levels, and expression of the hypothalamic CRF mRNA. Flavonoids can regulate CRF mRNA expression because flavonoids are able to modulate DNA binding activity of glucocorticoid receptors and cAMP, leading to phosphorylation of extracellular signal-regulated kinase ½ (ERK1 / 2) in the hypothalamus.[13]

The tannin content in the *Cassia alata* L. leaves ethanol extracts can activate mitogen-activated protein kinase (MAPK). Other studies have shown that MAPK is a family of protein kinases involved in the proliferation and division of Leydig cells. So that activation of MAPK by tannins can increase the number of Leydig cells in the interstitial tubules of the testes.[14] This can be a mechanism that prevented the negative effects of cell phone radiation that inhibits Leydig cell proliferation.[15]

The effect of *Cassia alata*L. Leaves Ethanol Extract on Testosterone

Research has found that cell phone radiation can reduce testosterone levels. Radiation of 1 mT, 50 Hz cell phone EMF for 85 days in male rats can reduce testosterone levels.[16] Cell phone radiation exposure for 60 minutes/day for 3 months significantly reduced serum testosterone levels in Wistar Albino rats compared with the control group.[17] Research showed that the decrease in testosterone levels in cell phone radiation is caused by a decrease in LH, so Leydig cells do not produce testosterone.[18]

In this study, the administration of *Cassia alata* L. leaves ethanol extracts can increase testosterone but this increase was statistically not significant. The minimal effect of *Cassia alata* L. leaves ethanol extracts on testosterone levels can be due to several causes. The first reason is that the dosage is not enough to increase testosterone levels, the second is less long research time to get significant results.

The dose used in this study was 10 mg/kgBW. This dose was chosen because based on the results of a preliminary study using 10, 20 and 30 mg/kgBW, the best results were 10 mg/kgBW to increase testosterone levels in the treatment group compared to the control group (result not shown). But the results of the main study with a larger sample size (18 rats/group), found the results of an increase in testosterone that was not statistically significant. Because of the large sample size, it is statistically possible that samples do not follow population distribution or are often called errors. This results in high standard deviations. Hence, when analyzed, the comparison will produce significant values that are not significant.[19] Previously, there had never been any research on the effects of *Cassia alata* L. leaves ethanol extracts on hormone levels, especially testosterone levels. Then the dose of this study cannot be compared.

Judging from the time of the study, there is also a possibility that the research time was not enough to increase testosterone levels significantly. This hypothesis is due to the fact that Leydig cells were significantly increased in the treatment group compared to the control group. Because Leydig cells are cells that produce testosterone, then theoretically testosterone levels will also be higher. However, based on insignificant results on testosterone levels there is a possibility that newly proliferating Leydig cells have not yet actively produced testosterone. Research shows that the differentiation of Leydig cells takes about 24 days and reaches a peak of testosterone production after 35 days.[20]

***Cassia alata*L. Leaves Ethanol Extract andAnti-Aging Medicine**

Aging is a natural process that will be experienced by every living thing and occurs gradually in all organs of the body. Decreased cell or organ function is one sign of the aging process which can result in the emergence of various pathological conditions that cause various complaints, degenerative diseases, decreased quality of life which will then end in death.[1]

The declining function of the endocrine system is an aging factor that can be triggered by various factors, one of which

is cell phone radiation. Cell phone radiation causes stress, thereby increasing cortisol which is a stress hormone. This causes interference with the HPG axis which that leads toa decrease in the number of Leydig cells and decreases testosterone levels. One indicator of the aging process is the presence of signs and symptoms caused by a decrease in testosterone,[1,2] so prevention of decreased testosterone caused by cell phone exposure can be used as an Anti-Aging Medicine (AAM) step.

In this study, oral administration of *Cassia alata*L. leaves ethanol extract prevented the decrease ofLeydig cells number but did not decreasedof testosterone level in male Wistar rats (*Rattus norvegicus*) exposed to cell phone radiation.*Cassia alataL.* leaves ethanol extracthas the potential to prevent aging. But this also needs to be further proven with a variety of doses and a longer study period to get significant testosterone results.

Previously, several other studies used a variety of plant extracts to prevent aging due to decreased testosterone levels induced by various causes of aging such as cigarette smoke, psychosocial stress, and excessive physical activity. When compared, *Cassia alata*L. leaves ethanol extract contained phenol (2975.10 mg / 100g GAE), flavonoids (1128.79 mg / 100g), tannin (4176.05 mg / 100g TAE), and total antioxidant (3465.65 mg / L) with IC50 of 372.8930 ppm.

Ashitaba leaves with higher bioactive compounds, namely flavonoids of 11523.66 mg / 100 QE, polyphenols of 3100.41 mg / 100 g GAE, tannins of 10569.44 mg / 100 g TAE, antioxidant capacity 28294.37 mg / L GAEAC, and IC 50% of 80.16 mg / L. The treatment of Ashitaba extract for 4 weeks can significantly prevent the decrease of testosterone in rats (Rattus norvegicus) male Wistar strain with psychosocial stress.[21] Other studies with lower phytochemical content, namely *Myrmecodia pendans*extract with a total phenol of 658.08 mg / 100g, flavonoids of 857.83 mg / 100g, tannins of 876.35 mg / 100g, total antioxidant capacity of 2141.62 mg / L and IC50 of 2008,0139 ppm for 14 days can increase serum testosterone levels in male Wistar rats exposed to cigarette smoke.[22]

5. Conclusion

It can be concluded that oral administration of *Cassia alata*L. leaves ethanol extract prevented the decrease ofLeydig cell number but did not decrease of testosterone level in male Wistar rats (*Rattus norvegicus*) exposed to cell phone radiation.

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