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RESEARCH ARTICLE

Effect of *Allium sativum*, *Curcuma mangga* and *Acorus calamus* Combination on the Uterus and Hormonal Profile in Rat Induced by Cisplatin

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ABSTRACT:

This research aimed to find out whether the combination of *Allium sativum*, *Curcuma mangga*, *Acorus calamus* extracts affect the weight of the uterus, uterus histology and estrogen-progesterone profile of cisplatin-induced rats. This experimental consist of seven treatments and four replications: untreated rats (K-), cisplatin 5mg/Kg BW (K+), dose extract of 50mg/Kg BW (P1), 75mg/Kg BW (P2), 100mg/Kg BW (P3), subur kandungan herbs (P4) and clomiphene citrate 0.9mg/Kg BW (P5). Uterine weight was obtained through weighing. Uterine histological slide were observed by the light microscope, whereas hormonal profile employed by ELISA method. The data were analyzed using the Anova continued with honest significant difference test. The result revealed that cisplatin induction significantly decreased the number of endometrial glands. The administration of extract combination significantly affected the uterine weight, endometrial thickness, number of endometrial glands and estrogen levels of cisplatin-induced rats. The dose of 75mg/Kg BW had the potential to increase the weight of the uterus, endometrial thickness and number of endometrial glands.

KEYWORDS: *Allium sativum*, *Curcuma mangga*, *Acorus calamus*, hormonal, uterine.

INTRODUCTION:

Some plants having the potential to overcome infertility problems are garlic (*Allium sativum*), temu mangga (*Curcuma mangga*) and jeringau (*Acorus calamus*). These three plants are the main components of herbal ingredients in traditional medicine to treat infertility. The presence of antioxidant activity and phytoestrogens was considered to be an essential factor for increasing fertility[1,2].

The presence of allicin compounds triggers antioxidant activity, flavonoid compounds namely kaempferol-3-O-β-D-glucopyranoside and isorhamnetin-3-O-β-D-glucopyranoside and polar phenolic compounds in *Allium sativum*, as well as phenolic compound contents of curcumin, chalcone, and flavanone and flavonoids in *Curcuma mangga*, also the presence of steroids, phenols, tannins and flavonoids compound in *Acorus calamus*[3,4,5]. Flavonoids could provide an antioxidant effect by preventing the formation of ROS or capturing free radicals *(free radical scavenger)* directly through the arrest of superoxide. Thus, the cell damage caused by an increase in free radicals can be reduced. The mechanism can then trigger regeneration and increase the proliferation of cells making up the reproductive organs[6,7].

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The previous studies reported that the combination of *Allium sativum* extract, *Curcuma mangga* and *Acorus calamus* significantly increased the thickness of the endometrial *layer*, myometrium and the number of uterine endometrial glands[1]. These results indicated that the extract combination was very effective in increasing fertility. Another experiment with same extract showed that level of estrogen and progesterone increased compared to rats given no extract combination (negative control). Meanwhile, a single dose of *Allium sativum* water extract was the optimal dose to increase the weight of the uterus of normal rats and it showed that the extract can increase estrogen and progesterone levels of heat-stressed rats[8]. This indicated the effectiveness of the combination dose of several medicinal plants compared to a single dose to increase fertility. Thus, research was needed to determine the effect of the combination of *Allium sativum*, *Curcuma manga* and *Acorus calamus* extract on the weight, uterine histology as well as the estrogen and progesterone profiles of cisplatin-induced rats.

MATERIAL AND METHODS:

Animal Models:

The animal models used in this study were (*Rattus norvegicus*) female Wistar strains white rats, which were ±2-3 months old, *had* 100-150g body weight and were fertile. Animal models were kept in cages made of plastic with wire mesh as the roof. Feed BR1 and drink were given *ad libitium*.

Materials:

Allium sativum, *Curcuma mangga*, and *Acorus calamus were* obtained and determined at UPT Materia Medica Batu, Indonesia. The process of making simplicia was carried out by the UPT Materia Medica Batu comprising the stages of harvesting, sorting, weighing, washing, slicing, molding, drying, oven drying, grinding until the packaging stage.

Extraction:

The extract combination was made by soaking 36g of garlic simplicia, 36g of *Curcuma mangga*, and 28g of jeringau in 70% ethanol for 24 hours at room temperature (maceration). The filtration was executed by using *Whatman* no.1 filter paper, and the pulp obtained was re-macerated using 70% ethanol. The stage was carried out three times until the filtrate was clear. The filtrate came out from the maceration was concentrated with a *rotary evaporator* at a temperature of 50ºC until concentrated extracts were obtained[4].

Cisplatin:

The induction of cisplatin to make rats became infertile which was carried out through *single-dose* intraperitoneal injection at a dose of 5mg/kg BW. Cisplatin was available as a solution with a composition of 50mg/50 mL[9].

Estrus Cycle Synchronization:

The estrous cycle synchronization was carried out before giving a treatment of a combination of extracts by injecting 0.2ml (10 IU) PMSG and hCG hormone. PMSG hormone injection was performed after ten days administration of cisplatin and hCG hormone injection, which was 48 hours after injecting PMSG hormone. Inoculation was carried out intraperitoneally[10].

Design and Treatment:

The research used complete randomized design with 7 treatments and 4 replications, which consist of: K- (Na CMC 0.5%), K+ (cisplatin 5mg/Kg BW + Na CMC 0.5%), P1 (cisplatin 5 mg/Kg BW + 50mg/Kg BW dose extract + Na CMC 0.5%), P2 (cisplatin 5mg/Kg BW + 75mg/Kg BW dose extract + Na CMC 0.5%), P3 (cisplatin 5mg/Kg BW + 100mg/Kg BW dose extract + Na CMC 0.5%), P4 (cisplatin 5mg/Kg BW + 75mg/Kg BW subur kandungan herb + Na CMC 0.5%), P5 (cisplatin 5mg/Kg BW + clomiphene citrate 0.9mg/Kg BW + Na CMC 0.5%).

Sample Collection, Hormonal Essay, and Histological Observation:

Blood samples were taken from the aorta to test estrogen and progesterone levels. The blood was incubated for 2 hours at room temperature and centrifuged at 1000rpm for 15 minutes. The supernatant obtained was separated from the pellet and was kept in a freezer at -70°C. The estrogen and progesterone levels were tested using the ELISA Kit (Bioassay Technology Laboratory). The uterine organ was taken and was washed using PBS and was cleaned with sterile paper. Uterus was weighed to obtain data on the uterine wet weight, then 10% formalin was added to make histological preparations with HE staining. Further, the thickness measurements of each layer were carried out in the four areas using *Image Raster Software.*

Data analysis:

The data obtained were tested for normality and homogeneity, followed by the One-Way Analysis of Varians test. If there are significant differences ($p < 0.05$), the statistical test is continued with honest significant difference (HSD) test.

RESULTS AND DISCUSSION:

This study shows the results of the study as follows:

Table 1. The thickness of rat's uterus, endometrium, myometrium, perimetrium, and weight

Treatment	Average Thickness ± SD (µm)			Average Uterus Weight ± SD (mg)
	Endometrium	Myometrium	Perimetrium	
K- (Normal rats)	490.29 ± 39.49 [b]	172.20 ± 41.29	148.76 ± 35.32	341.5 ± 99.48 [b]
K + (Cisplatin)	421.54 ± 11.92 [ab]	140.72 ± 27.13	141.88 ± 17.16	361.25 ± 60.52 [b]
P1 (Dose of 50 mg/Kg BW)	360.72 ± 43.34 [a]	118.51 ± 10.06	119.97 ± 6.10	213.25 ± 91.35 [a]
P2 (Dose of 75 mg/Kg BW)	437.44 ± 42.29 [ab]	148.36 ± 30.24	143.28 ± 24.46	379.50 ± 77.36 [b]
P3 (Dose of 100 mg/Kg BW)	428.49 ± 42.65 [ab]	145.46 ± 4.87	142.44 ± 27.28	307.00 ± 27.53 [ab]
P4 (Subur kandungan herb)	478.66 ± 58.11 [b]	155.94 ± 14.30	144.36 ± 25.56	310.00 ± 11.07 [ab]
P5 (Clomiphene citrate)	420.44 ± 51.31 [ab]	132.97 ± 13.52	130.52 ± 18.22	203.75 ± 11.70 [a]

Table 1 revealed that induction of cisplatin had not been able to influence the decrease in uterine weight compared to K-. The combination of *Allium sativum*, *Curcuma mangga* and *Acorus calamus* at P1, P3 and P4 could not increase the weight of the rat's uterus induced by cisplatin. Whereas P2 could increase the weight of the rat's uterus induced by cisplatin, but it was not significant. The results of this study were comparable which affirm that the weight of normal rats uterine could increase after the administration of a single dose of 200 mg/Kg BW extract of *Allium sativum* water for 28 days of treatment[8]. The weight of the uterus was strongly influenced by the thickness of the endometrium and secretions produced by the uterine gland[11]. This study indicated that endometrial thickness and the number of endometrial glands were not directly proportional to the weight of the uterus because the uterus was an organ consisting of many constituent tissues. The increased weight of rat's uterus in P2 was caused by phytoestrogen compounds and antioxidant activity in the combination of *Allium sativum*, *Curcuma mangga*, and *Acorus calamu*s. A combination of extracts administration was presumed to have a synergistic effect, so a beneficial effect was obtained. Flavonoids, isoflavone, and triterpenoid were known as estrogenic causing endogenous estrogen-like effects[12].

Endometrial thickness in the group given cisplatin was lower than K+, but it was not significant due the main gonadotoxic effect of cisplatin was not on the uterine endometrial lining, but on the ovary[9]. The administration of the combined extract of *Allium sativum*, *Curcuma mangga* and *Acorus calamus* on P1 could not increase the endometrial thickness of rats induced by cisplatin. P2 and P4 could increase the endometrial thickness of rats induced by cisplatin, but it was not significant. P3 tended to reduce endometrial thickness compared to P2. This study showed that the combination dose of extract given influenced the endometrial thickness. It was probably caused by flavonoids and triterpenoids contained in the combination of *Allium sativum*, *Curcuma mangga* and *Acorus calamus* that acted like estrogen[1]. Estrogen in high concentrations provides negative feedback on the hypothalamic-pituitary-ovarian axis. Thus, it inhibited the release of *Luteinizing Hormone* (LH) and *Follicle Stimulating Hormone* (FSH). The decreasing LH and FSH could reduce the number of follicles leading to decreasing production of estrogen, and it inhibited endometrial cell proliferation[13]. Meanwhile, active compounds such as flavonoids in extract combination could increase the thickness of the endometrium through its antioxidant activity[14].

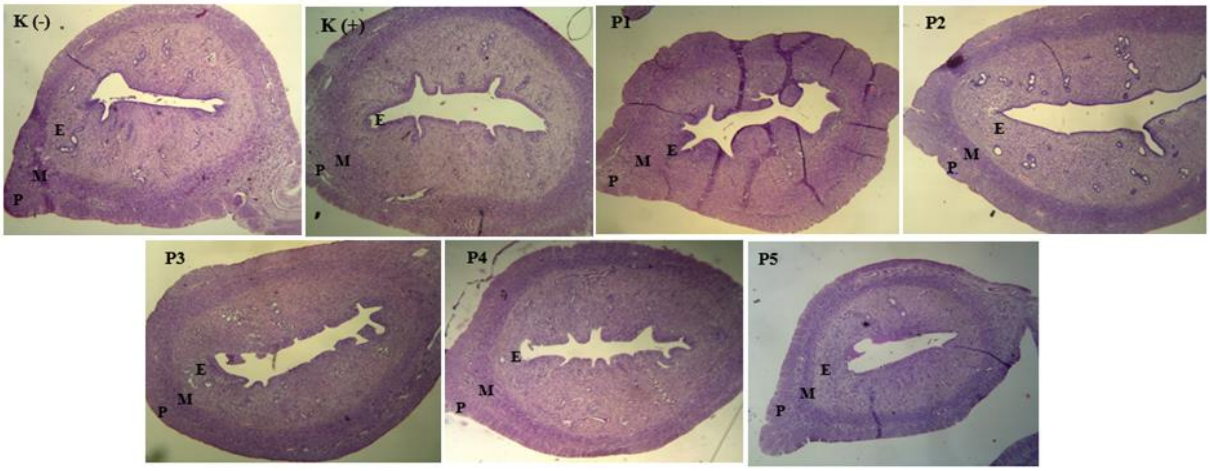

Figure 1. Histology of rat's uterus with HE staining at a 40x magnification after treatment. E (Endometrium), M (Myometrium), P (Perimetrium); K- : Untreated rats; K+: Cisplatin; P1: Dose of 50 mg/Kg BW; P2: Dose of 75 mg/Kg BW; P3: Dose of 100 mg/Kg BW; P4: Subur kandungan herb; P5: Standard drug (clomiphene citrate).

Notes: K-: Normal (Untreated rats) ; K+: Cisplatin; P1: Dose of 50 mg/Kg BW; P2: Dose of 75 mg/Kg BW; P3: Dose of 100 mg/Kg BW; P4: Subur kandungan herb; P5: Standard drug (clomiphene citrate)

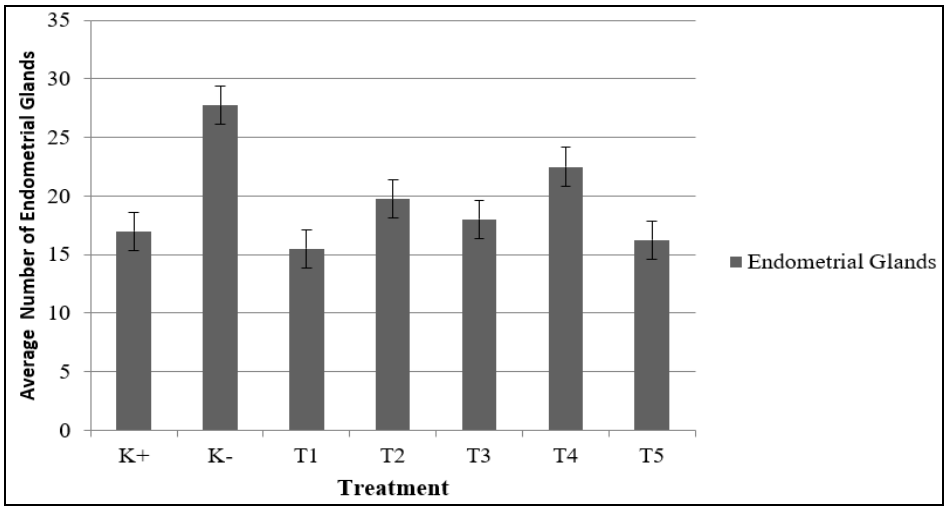

Figure 2. The number of endometrial glands of cisplatin-induced rats after treatment. Description: K-: Normal rats, K+: Cisplatin, P1: Dose of 50 mg/Kg BW, P2: Dose of 75 mg/Kg BW, P3: Dose of 100 mg/Kg BW, P4: Subur kandungan herb and P5: Clomiphene citrate

The number of rat's endometrial glands (Figure 2.) was significantly lower than K+ group compared to K- group, indicated that an increase in free radicals due to the induction of cisplatin could help to regenerate the endometrial gland cells cisplatin was proven to cause apoptosis in endometrial primary cell cultures[15]. P1 dose treatment could not increase the number of endometrial glands. P2 could increase the number of endometrial glands of cisplatin-induced rats, but it was not significant. P3 tended to decrease the amount of endometrial glands compared to P2. P4 was considered as the best treatment because it significantly increased the number of endometrial glands. The previous research reported that the administration of subur kandungan herb could significantly increase the number of normal rat's endometrial gland compared to those who were not given herbal medicine[1]. Subur kandungan herb contained main ingredients such as *Allium sativum*, *Curcuma mangga* and *Acorus calamus* which was proven to provide active compounds that can act as antioxidants and phytoestrogens. Exogenous antioxidants might be extracted from plants containing phenolic compounds such as polyphenols, flavonoids, alkaloids, and curcuminoids[4]. Antioxidant activity of secondary metabolites could protect cells from oxidative damage by neutralizing reactive oxidants and helping the cell proliferation process[16,17]. Meanwhile, phytoestrogens compounds such as isoflavones and triterpenoids worked in the same way as estrogen, which bound estrogen receptors to form ligand-receptor complexes, then induced the expression of genes responsive to estrogen. Thus, it triggered cell proliferation[18].

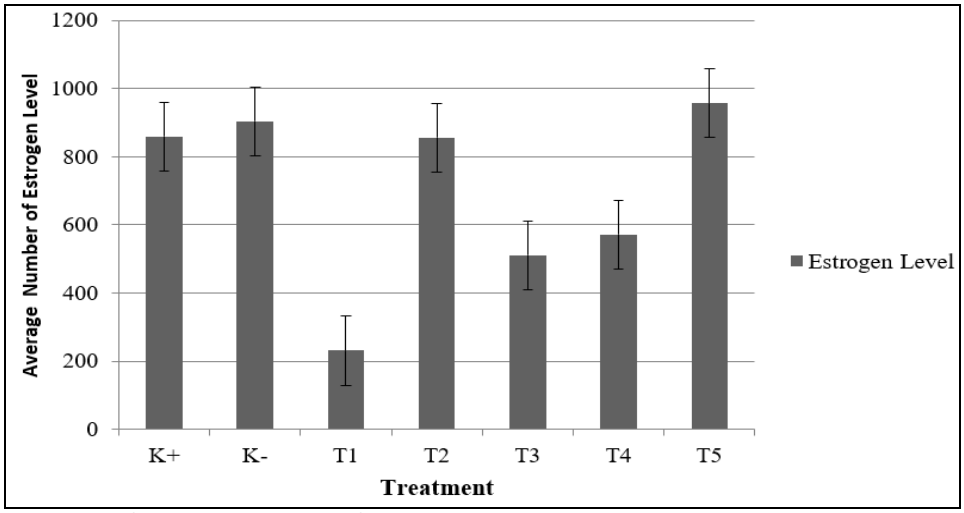

Figure 3. The estrogen level of cisplatin-induced rats after treatment. Description: K-: Normal rats, K+: Cisplatin, P1: Dose of 50mg/Kg BW, P2: Dose of 75mg/Kg BW, P3: Dose 100 mg/Kg BW, P4: Subur kandungan herbal and P5: Clomiphene citrate.

The estrogen levels of cisplatin-induced rats (Figure 3.) could not be increased with all combinations of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus*. This result was not comparable with the previous research that the giving of Cisplatin, Subur kandungan herb, Clomiphene citrate and composition of *Allium sativum*, *Curcuma Mangga* and *Acorus calamus* significantly increased the endometrial thickness compared to untreated rats (negative control). It was presumed that ovarian oxidative stress was occurred caused by cisplatin induction although it was not significant. Thus, compounds like *allicin*, curcumin, flavonoids, and alkaloids as antioxidants in extract combination, were considered unable to neutralize free radicals in the ovary. It then caused estrogen synthesis and production to decrease[4]. It was known that triterpenoid saponin compounds in glycans containing steroid diosgenin which could be synthesized into estrogen through a series of chemical reactions[15]. However, estrogen synthesis, after being given a combination of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus*, have not been able to increase estrogen levels of rats induced by cisplatin.

CONCLUSION:

It can be concluded that the induction of cisplatin as a gonadotoxic agent to make rats infertile triggers the decrease of the number of endometrial glands significantly. Next, the administration of the combination of *Allium sativum*, *Curcuma manga* and *Acorus calamus* with the dose of 75mg/Kg BW in cisplatin-induced rats had the potential to increase the weight of the uterus, endometrial thickness and number of endometrial glands.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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