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Evaluating the Effect of Polyherbal Extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* on Immunomodulation and Ovarian Activity in Cisplatin-Induced Rats

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ABSTRACT

Background: Exogenous antioxidants come from synthetic or natural ingredients are often used to reduce oxidative stress caused by free radicals. *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* are known to have a good number of antioxidants that has many benefits, such as antimicrobial, antiviral, anti-inflammatory and immunomodulatory properties. **Aim:** This study aims to determine the polyherbal effect of *Allium sativum* (garlic), *Curcuma mangga* (mango ginger), and *Acorus calamus* (sweet flag) on ovarian antioxidant activity, follicles profile, and immunomodulation activity in cisplatin-induced rat.

Materials and Methods: Fourty-two female rats (*Rattus norvegicus*) of three-month-old (180-230 gBW) were used and were divided into seven treatment groups. All experimental groups except the negative control (C-) were intraperitoneally injected with 5 mg of cisplatin. After ten days of cisplatin induction, each group (T1, T2, T3, T4, and T5) was orally given several type of treatments such as polyherbal dose of 50, 75, 100 mg/kg BW, Subur Kandungan™, and clomiphene citrate dose of 75 and 0.9 mg/kg BW, respectively. These treatments were given for 15 days. The activity of ovarian superoxide dismutase (SOD) in the polyherbal groups was significantly increased compared to the C- group (p <0.05).

Results and Discussion: The T4 group showed the highest SOD activity followed by T5, T2, T3, C+, T1, and C-, respectively, whereas the ovarian MDA activity revealed the opposite pattern. Additionally, we showed that the administration of polyherbal increased the number of primary follicles, secondary follicles, tertiary follicles, de Graf follicles, and ovulation significantly compared to C+ group. The relative number of $CD4^+TNF\alpha^+$ and $CD11b^+TNF\alpha^+$ were significantly increased in polyherbal groups compared to C- group (p <0.05). The C+ group showed the highest expression of $CD4^+TNF\alpha^+$ followed by T5, T3, T4, T1, and C-, while the T4 group expressed the highest $CD11b^+TNF\alpha^+$ (p <0.05).

Conclusion: In sum, the polyherbal extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* at the dose of 75 mg/kg BW produced the highest ovarian antioxidant, ovarian follicles profile, and immunomodulatory activities in cisplatin-induced rats.

Keywords: *Acorus calamus*, *Allium sativum*, cisplatin, *Curcuma mangga*, immunomodulation, ovarian.

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INTRODUCTION

Cisplatin is a gonadotoxic intermediary agent which has toxic effects on primordial follicles. Interestingly, the cisplatin can stop the regeneration of primordial follicles followed by triggering damage or dysfunction in the ovaries which directly lead to infertility. Several adverse effects of cisplatin drive to a premature ovarian failure (POF), estrous cycle failure, and decreased anti-Mullerian hormone [1]. In addition, cisplatin could increase free radicals through the induction of glucose-6-phosphate dehydrogenase and hexokinase activities, thereby it could inhibit the activity of antioxidant enzymes. Furthermore, increased the free radicals in cells cause imbalances in the immune system. One of the main components of the immune system is T cells, as adaptive immunity [2, 3]. Oxidative stress caused by free radicals can be prevented and the body's immunity can be improved by administering antioxidants [4,5].

Exogenous antioxidants come from synthetic or natural ingredients [6]. Plants are one of the natural ingredients that are rich of antioxidants potency [7]. Garlic has many benefits, such as antimicrobial, antiviral, and immunomodulatory properties. Garlic is rich in organosulfur, tannins, and glycosides that take part in biological effects [8]. Moreover, mango ginger contains curcuminoid, glycosides, and anthraxon [9]. Mango ginger has also been reported as having antiinflammatory, immunomodulatory, and nitric oxide inhibitory activities [10]. Furthermore, the sweet flag contains alkaloids, triterpenoids, and essential oils, which are useful as anti-inflammation, immunomodulator, and antifungal [11]. Based on the above background, it is important to evaluate the effect of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* in increasing ovarian and immunomodulation activities in cisplatin-induced rat.

MATERIALS AND METHOD

All treatments procedures have been approved by Health Research Ethics Committee (KEPK), Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University of Malang (approval reference number: 016/EC/KEPK-FKIK/2018). Materials used in this study were polyherbal of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* (UPT Materia Medica Batu, Indonesia), Subur Kandungan™ (PJ. Ribkah Joko Tole, Madura, Indonesia) and clomiphene citrate (Sanbe Farma, Indonesia), SOD and MDA kit (Chenguang Biotech Group Co., Ltd. China), fixation buffer (Biolegend), intracellular staining permeabilization wash buffer (Biolegend), FITC-conjugated anti-rat CD4 antibody, PE-conjugated anti-rat TNF-α antibody (Bioss), FITC-conjugated anti-rat CD11b antibody (Novus).

Fourty-two female rats (*Rattus norvegicus*) of three-month-old (180-230 gBW) were used. Further, they were induced with a single dose of cisplatin 5 mg/kg BW [1]. After ten days, the estrous cycle was synchronized with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) of 10 IU 0.2 mL per rat. The hCG injection was carried out 48 hours after the PMSG injection. The next day, the estrous cycle was checked to identify the start of the estrous cycle using the vaginal smear method.

A total of 36 g *Allium sativum*, 36 g *Curcuma mangga*, and 28 g *Acorus calamus* were soaked in 70% ethanol for 24 h at room temperature (maceration) referring to our previous study [12]. The research used Complete Randomized Design with seven treatment groups and six replications, consisting of C-: rats without any treatment, C+: rats induced by cisplatin only, T1, T2, T3, T4 and T5: rats induced by cisplatin and given 50, 75, 100 mg/kg BW polyherbal, 75 mg/kg BW Subur Kandungan™, and 0.9 mg/kg BW clomiphene citrate, respectively.

The determination of MDA and SOD activity was adapted from other previous studies [13, 14]. Histology preparation of ovarium was carried out by adapting the method of El-Zahraa and Elhafez [15]. The spleenocyte were isolated by crushing the spleen in PBS solution for further analysis via FACSCalibur™ [16]. In addition, the data from this study were analyzed with normality and homogeneity tests followed by the analysis of variance (ANOVA) and Duncan's Multiple Range test. All tests used SPSS 15 (SPSS Inc., USA). The differences were considered significant when p <0.05.

RESULTS AND DISCUSSION

The Activity of Ovarian SOD and MDA

The activity of ovarian SOD in the experimental groups significantly increased compared to C- group (p <0.05). The highest to lowest SOD activities were found consecutively at T4, T5, T2, T3, C+, and T1. In addition, the highest to lowest ovarian MDA activities were found in the following treatments successively T1, C+, T3, C-, T5, T2, and T4. Importantly, the polyherbal treatment could increase the antioxidant activity of the ovaries, whereas lipid peroxidase showed the opposite pattern (Table 1). The SOD activity in ovarian showed an increase in the treatment groups.

Natural antioxidants activity of the polyherbal extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* come from the bioactive compounds [8, 9]. Moreover, Badr and Al-Mulhim (2014) reported that *Allium sativum* (200 mg/kg BW) could significantly cure gastric mucosal damage, normalized increase of MDA, MPO (myeloperoxidase) and TNF-α also stabilized decreasing of tGSH, SOD, and CAT values [17]. Flavonoids are exogenous antioxidant compounds that are proven to be able to ward off free radicals by releasing hydrogen atoms from their hydroxyl groups [7]. Activation of endogenous antioxidant enzymes was carried out by flavonoid compounds, then SOD as a powerful antioxidant will activate nuclear factor erythroid 2 (NRF2) [18]. NRF2 is a protein in cells that works by the ECH association protein 1 (Keap1) repressor, which is then activated by oxidative stress [19]. MDA is toxic, which causes the breaking of the carbon chain of fatty acids in the lipid peroxidation process [20]. Cisplatin increases MDA by decreasing antioxidant levels and inducing glucose-6-phosphate dehydrogenase activity [2].

Table 1. The activity of ovarian SOD and MDA after treatments.

Treatment	The activity of SOD (X±SD) (u/mL)	The activity of MDA (X±SD) (u/mL)
C-	2.66^{a}±0.15	14.25^{bc}±0.76
T1	3.02^{b}±0.12	18.75^{d}±2.55
T2	3.73^{d}±0.07	12.25^{ab}±1.39
T3	3.58^{c}±0.04	14.25^{bc}±1.35
T4	4.12^{e}±0.06	10.50^{a}±0.91
T5	4.07^{e}±0.05	13.50^{bc}±1.18
C+	3.53^{c}±0.03	16.50^{cd}±1.26

*Different alphabetic superscripts in the same column indicated a significant difference (p <0.05)

Ovarian Follicles Profile

In this study, we showed that the administration of polyherbal increased the number of primary follicles, secondary follicles, tertiary follicles, de Graf follicles, and ovulation significantly compared to C+ group (p <0.05) (Table 2 and Figure 1). The highest number of primary follicles and ovulation was obtained by T2. The induction of cisplatin without any treatment produced the lowest number of secondary follicles and tertiary follicles and the highest of atretic follicles. In the histological image of C+, there were quite a lot of atretic follicles, and their number decreased significantly (p <0.05) in other treatments (Figure 1). Injection of cisplatin caused oxidative damage to the ovaries. The effect of cisplatin induction on rats in this study caused a decrease in follicular development and the number of ovulations compared to mice without cisplatin induction (Table 2). According to Akunna *et al*. (2017) and Aksoy *et al*. (2015), cisplatin induction causes the number of primordial, secondary, and tertiary follicles are decreasing while the number of atretic follicles increases [1, 2]. Cell death or apoptosis can occur in primordial follicular oocytes that cause infertility [21].

Table 2. Ovarian follicle profile after treatment.

Treatment	Follicle Number					
	Primary Follicle	Secondary Follicle	Tertiary Follicle	de-Graf Follicle	Ovulation Number	Atretic Follicle
C-	18.50^{bc}±1.29	6.00^{ab}±0.16	3.00^{ab}±0.82	0.75^{a}±0.10	10.25^{ab}±1.63	0.00^{a}±0.00
T1	11.25^{a}±2.75	7.25^{ab}±0.35	2.75^{ab}±0.26	2.00^{ab}±0.21	8.75^{ab}±1.77	1.50^{bc}±0.58
T2	19.25^{c}±2.22	5.00^{ab}±0.32	4.25^{ab}±0.75	2.25^{ab}±0.16	11.00^{b}±1.16	1.00^{bc}±0.00
T3	11.50^{a}±1.80	8.25^{b}±0.71	3.00^{ab}±0.83	3.00^{b}±0.82	6.75^{ab}±1.71	2.00^{d}±0.00
T4	13.25^{a}±1.11	5.75^{ab}±0.26	4.50^{ab}±0.08	3.00^{b}±0.83	9.50^{ab}±1.65	1.75^{d}±0.50
T5	14.75^{a}±2.63	9.50^{b}±0.29	5.50^{b}±0.89	2.75^{b}±0.26	5.75^{a} ±1.22	0.75^{b}±0.50
C+	14.25^{ab}±2.99	3.00^{a}±0.83	1.50^{a}±0.29	1.50^{ab}±0.18	8.50^{ab}±1.65	4.50^{e}±0.58

*Different alphabetic superscripts in the same column indicated a significant difference (p <0.05)

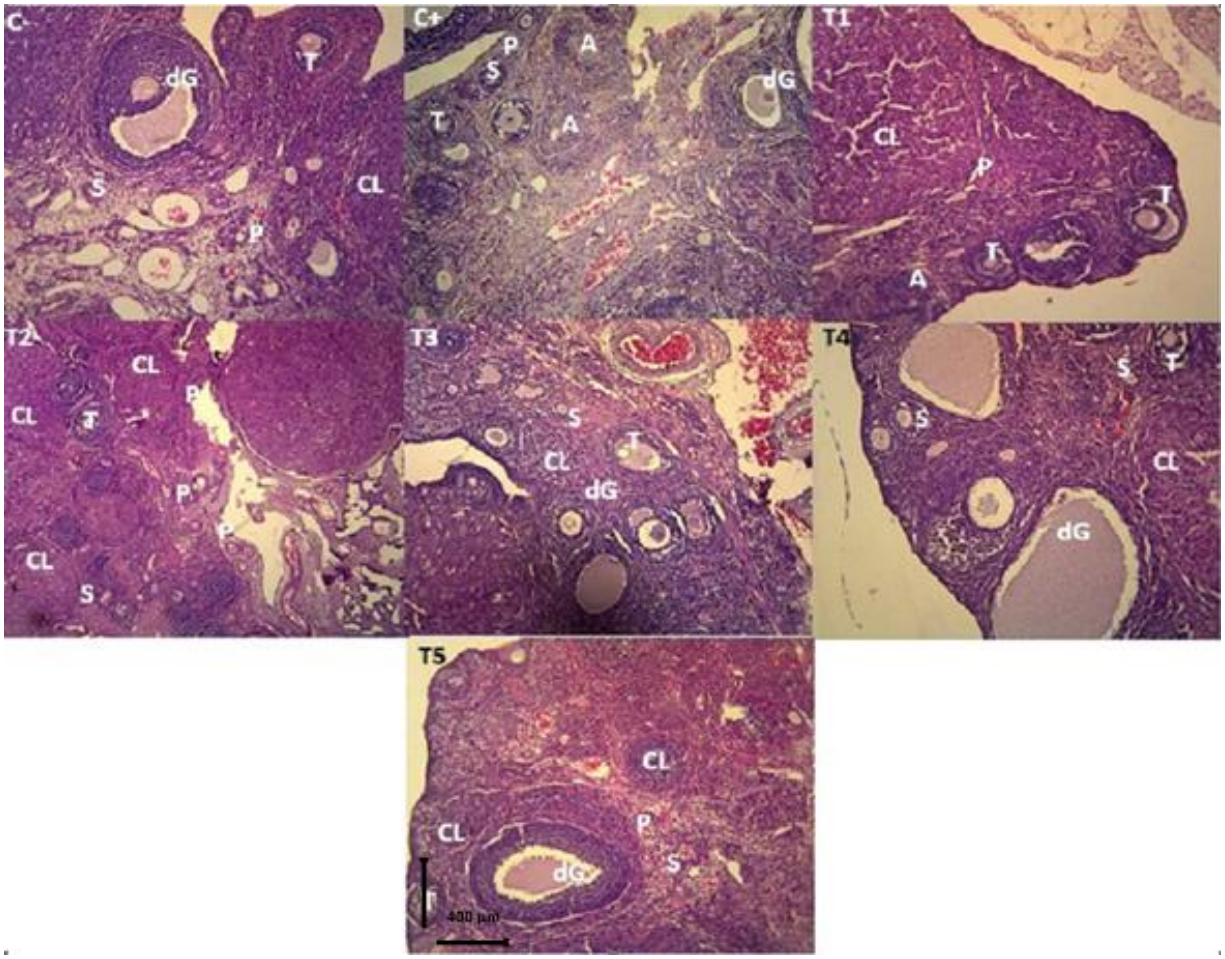

Figure 1. Histological profile of ovarian follicles after treatment (M: 400×). P: primary follicle, S: secondary follicle, T: tertiary follicle, dG: de Graf follicle, A: atretic follicle.

Polyherbal Treatment Modulates Pro-Inflammatory Cytokine

In this present study, we revealed that the administration of polyherbal significantly increased the amount of pro-inflammatory cytokine in treatment groups compared to C- group (p <0.05). On the other hand, polyherbal increased pro-inflammatory $CD11b^{+}TNF\alpha^{+}$ cells response in treatment groups compared to C- group (Figure 2). T4 could promote the number of $CD11b^{+}TNF\alpha^{+}$ cells followed by C+ and T5, and these treatments are significantly different from the others (p <0.05). C- group showed the lowest number of $CD11b^{+}TNF\alpha^{+}$ cells, not significantly different from T1 but different from T3 and T2. In our finding, the administration of polyherbal in T2 and T3 significantly increased pro-inflammatory cytokines compared to C-, indicated by increasing TNF-α (Figure 2). The polyherbal reduced inflammation level of the cisplatin-induced rat in a dose-dependent manner. The results showed that polyherbal reduced the production of pro-inflammatory mediators such as TNF-α. The relative numbers of $CD4^{+}TNF\text{-}\alpha^{+}$ and $CD11b^{+}TNF\text{-}\alpha^{+}$ cells significantly decreased in polyherbal-cisplatin induced rat groups compared to the C+ group (Figure 2).

The ability of flavonoids and other active compounds found in polyherbal plays an active role in increasing the secretion of TNFα from $CD4^{+}$ T cells and $CD11b^{+}$

macrophage cells in the treatment group especially at the dose of 75 mg. Treatment with Subur Kandungan™ resulted in the highest number of $CD11b^{+}TNF\alpha^{+}$ cells not significantly different from clomiphene citrate and cisplatin (Figure 2). Very high inflammatory cell proliferation could cause lipid peroxidation so that it becomes pro-oxidant [22].

Garlic plays a role in healing several pathologies, and its effect on immune system components is associated with the pro-inflammatory state of metabolic syndrome [23]. In addition, *Curcuma mangga* revealed the presence of steroids, terpenoids, and curcumin that demonstrated the immunostimulatory effect on phagocytosis ability [9]. *Acorus calamus* extract inhibited the production of NO, IL-2, TNFα, CD25, mitogen, antigen-stimulated human PBMCs and inhibited the growth of several cell lines of mouse and human [24].

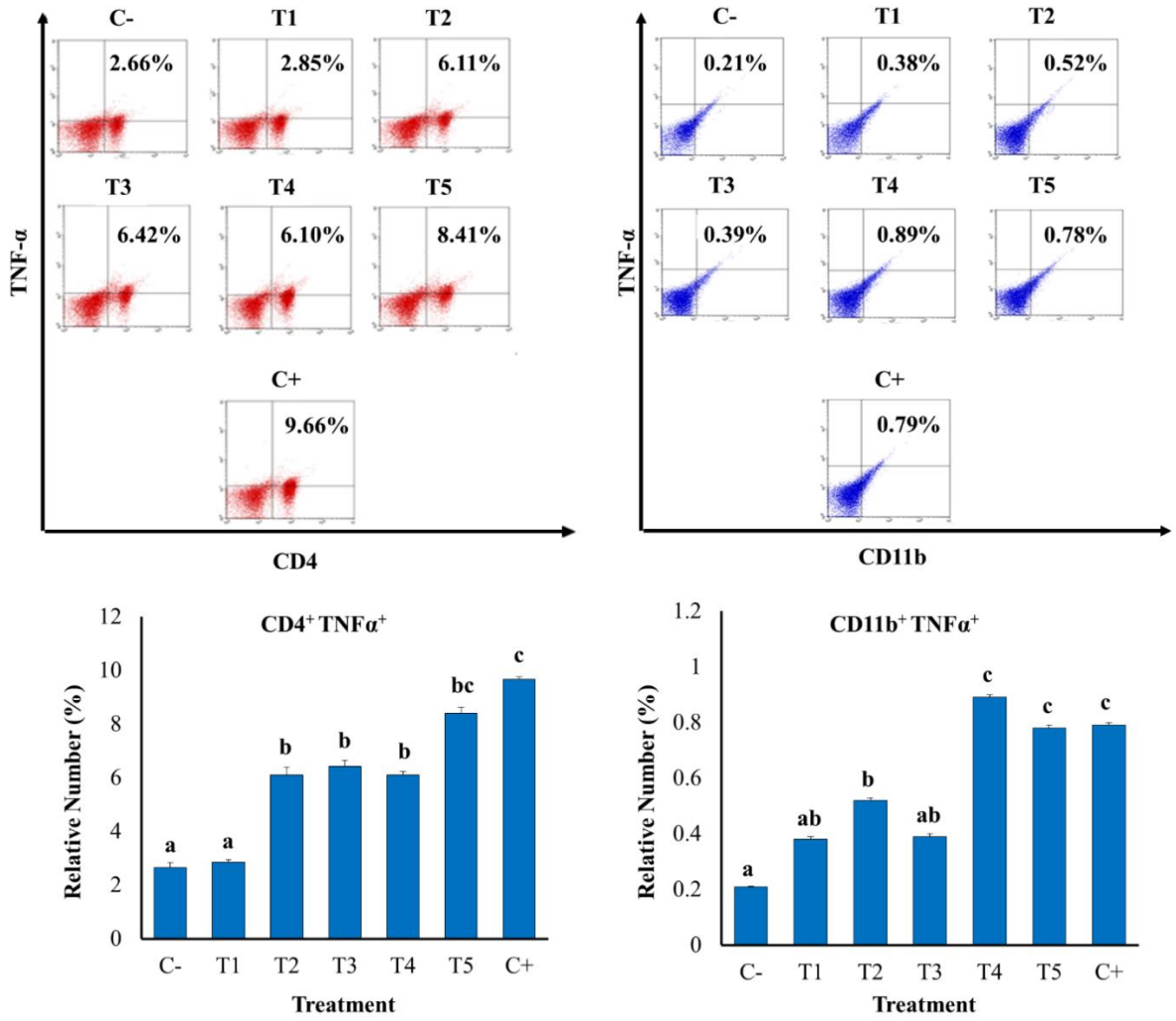

Figure 2. The immunomodulation profile of polyherbal extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* toward the relative number of CD4+ TNFα+ and CD11b+ TNFα+. Different alphabetic superscripts indicated a significant difference ($p < 0.05$).

CONCLUSION

In sum, we suggested that the polyherbal extract of *Allium sativum*, *Curcuma mangga*, and *Acorus calamus* at the dose of 75 mg/kg BW produced the highest ovarian antioxidant, ovarian follicles profile, and immunomodulatory activities in cisplatin-induced rats.

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REFERENCES

1. Akunna GG, Nwafor J, Egwu OA, et al. Cisplatin-induced ovarian cytotoxicity and the modulating role of aqueous zest extract of *Citrus limonium* (AZECL) in rat models. J Tradit Med Clin Natur. 2017; 6:1-7.
2. Aksoy AN, Kabil-Kucur S, Batmaz G, et al. The role of antioxidant activity in the prevention and treatment

of infertility caused by cisplatin in rats. Gynecol Obstet Invest. 2015; 79:119-125.
3. Chen H, Wen F, Zhang X, et al. Expression of T-helper-associated cytokines in patients with type 2 diabetes mellitus with retinopathy. Mol Vis. 2012; 18:219-226.
4. Susilo RJK, Winarni D, Husen SA, et al. Hepatoprotective effect of crude polysaccharides extracted from *Ganoderma lucidum* against carbon tetrachloride-induced liver injury in mice. Vet World. 2019; 12:1987-1991.
5. Ansori ANM, Fadholly A, Hayaza S, et al. A review on medicinal properties of mangosteen (*Garcinia mangostana* L.). Res J Pharm Technol. 2020; 13:974-982.
6. Hayaza S, Istiqomah S, Susilo RJK, et al. Antidiabetic activity of ketapang (*Terminalia catappa* L.) leaves extract in streptozotocin-induced diabetic mice. Indian Vet J. 2019; 96:11-13.
7. Ansori ANM, Susilo RJK, Hayaza S, et al. Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. Iraqi J Vet Sci. 2019; 33:13-19.
8. Ali M and Ibrahim IS. Phytochemical screening and proximate analysis of garlic (*Allium sativum*). Inorg Chem. 2019; 4:478-482.
9. Kamazeri TS, Samah AT, Taher OA, et al. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. Asian Pac J Trop Med. 2012; 5:202-209.
10. Yuandani, Yuliasmi S, Satria D, et al. Correlation between the phytochemical constituents of *Curcuma mangga* and its immunomodulatory effect. Rasayan J Chem. 2019; 12:1-6.
11. Muchtaromah B, Ahmad M, Sabdoningrum EK, et al. Phytochemicals, antioxidant and antifungal properties of *Acorus calamus*, *Curcuma mangga*, and *Allium sativum*. KnE Life Sci. 2011; 3:93.
12. Muchtaromah B, Kiptiyah and Adi TK. Transaminase enzyme and liver histological profile of mice administered extract of pegagan (*Centella asiatica* (L.) Urban). Trop Anim Sci J. 2011; 34:88-92.
13. Husen SA, Wahyuningsih SPA, Ansori ANM, et al. The effect okra (*Abelmoschus esculentus* Moench) pods extract on malondialdehyde and cholesterol level in STZ-induced diabetic mice. Eco Env Cons. 2019a; 25: S50-S56.
14. Husen SA, Wahyuningsih SPA, Ansori ANM, et al. Antioxidant potency of okra (*Abelmoschus esculentus* moench) pods extract on SOD level and tissue glucose tolerance in diabetic mice. Res J Pharm Technol. 2019b; 12:5683-5688.
15. El-Zahraa F and Elhafez A. A histological, histochemical and ultrastructural characterization of uterine vessels at early stages of pregnancy. Histol Histopathol. 2018; 2:41-47.
16. Hartati FK, Widjanarko SB, Widyaningsih TD, et al. Anti-Inflammatory evaluation of black rice extract inhibits TNF-α, IFN-γ and IL-6 cytokines produced by immunocompetent cells. Food Agr Immunol. 2017; 28:1116-1125.
17. Badr GM and Al-Mulhim JA. The protective effect of aged garlic extract on nonsteroidal anti-inflammatory drug-induced gastric inflammations in male albino rats. Evid-Based Complementary Altern. Med. 2014; 1-9.
18. Kim HM, Han JW and Chan JY. Nuclear factor erythroid-2 like 1 (NFE2L1): Structure, function and regulation. *Gene*. 2016; 584:17-25.
19. Kobayashi H, Wang C and Pomper KW. Phenolic content and antioxidant capacity of pawpaw fruit (*Asimina triloba* L.) at different ripening stages. Hortic Sci. 2008; 43:268-270.
20. Samik A and Safitri E. Potency of mycotoxin binders on MDA level, expressions of caspase 9 and caspase 3 in the uterus of mice exposed to zearalenone. Iraqi J Vet Sci. 2017; 31:29-33.
21. Altuner D, Gulaboglu M, Yapca O, et al. The effect of mirtazapine on cisplatin-induced oxidative damage and infertility in rat ovaries. Sci World J. 2013;1-6.
22. Nicole CBSP. Role of flavonoids in oxidative stress. Curr Top Med Chem. 2005; 1:569-590.
23. Trio PZ, You S, He X, et al. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. Food Funct. 2014; 5:833-844.
24. Mehrotra S, Mishra KP, Maurya R, et al. Anticellular and immunosuppressive properties of ethanolic extract of *Acorus calamus* rhizome. Int Immunopharmacol. 2003; 3:53-61.