

## **Taraxacum officinale Leaves Ethanolic Extract as Immunostimulatory Agent For Reducing Side Effect of Doxorubicin in Sprague Dawley Rats**

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### **Abstract**

Doxorubicin as chemotherapeutic agent causes immunosuppressive. The aim for this study to determine the effect of ethanolic extract of *Taraxacum officinale* (ETO) in immunity system of Sprague Dawley rat that induced by doxorubicin to observe the profile of immunity cells. Sprague Dawley rats were divided into five groups each groups contain five rats : control doxorubicin group, doxorubicin dose 4,67 mg/kgBW+ ETO dose 1000 mg/kgBW, doxorubicin dose 4,67 mg/kgBW+ ETO dose 500 mg/kgBW, control extract group, and without treatment. Then the number of leukocytes, lymphocytes and neutrophils were analyzed by hematology analyzer, whereas CD8+ T lymphocytes by flowcytometry. Results showed groups of doxorubicin combined with ETO dose 1000 mg/kgBW and 500 mg/kgBW increased the number of leukocytes, lymphocytes, neutrophils, cytotoxic CD8 + T cells T cells compared to control doxorubicin group. These data presents that etanolic extract of Jombang leaves has immunostimulatory activity and potential as co-chemotherapy agents. Molecular mechanism underlying it's immune activity need to be explored in detail.

**Keywords** : co-chemotherapy, doxorubicin, immunostimulatory, in vivo *Taraxacum officinale*,

### **INTRODUCTION**

Cancer is a disease caused by the growth of body tissue cells that abnormal and uncontrollable. The main treatment is mostly done to treat cancer such as surgery, radiation, and chemotherapy (Siahaan, 2005). However, prolonged use of chemotherapy can cause weakening of the immune system (Patel *et al.*, 2006).

The use of doxorubicin as an chemotherapy agent is considered effective (Frias *et al.*, 2009). However, the use of doxorubicin was thinking about some side effects such as causing cardiotoxicity (Frias *et al.* 2009; Bustova, 2009), susceptible to disease, hair loss, and sore throat. In addition, doxorubicin also affects the immune system by reducing the expression of IL- $\alpha$ , interferon production  $\gamma$ , natural killer cells (NK), lymphocyte proliferation, and the ratio of CD4 + / CD8 + (Zhang *et al.*, 2005). Therefore, we need a compound that works

synergy with doxorubicin, but can reduce the immunosuppressive side effects of doxorubicin.

Today, we've discovered various materials which otherwise could increase the body's resistance to a disease called as immunostimulant. Ethanolic extract of *Taraxacum officinale* leaves (ETO) is reported able to increase the secretion of TNF- $\alpha$  and IL-1 (Kim *et al.*, 2000; Koo *et al.*, 2004). TNF- $\alpha$  works together with IL-1 as functional non-specific immunity (Baratawidjaja, 2006). Jeon *et al.* (2007) also figured out that ETO is able to influence the antiinflammatory response through the inhibition of synthesis of nitric oxide (NO) and inhibition of COX-2 expression. Therefore, ETO can be expected used as immunostimulatory for combination with doxorubicin (co-chemotherapy).

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The aim for this study to find alternative safe co-chemotherapy in cancer therapy and determine the effect of ETO on immune cells in Sprague Dawley rats induced by doxorubicin

## MATERIALS AND METHODS

### *Taraxacum officinale* Extraction

*Taraxacum officinale* leaves powder was obtained and determined by B2P2TO2T (Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional) Tawangmangu, Central Java. Powder of *Taraxacum officinale* leaves (1000 g) was extracted with 70% ethanol (10 l) (1:10 b/v). Ethanolic filtrate then concentrated by a rotary evaporator, followed by freeze drying to obtain viscous extract (150.7 g).

### Animals

Female, 60 days old, Sprague Dawley, weighed from 90 to 120 g were purchased from UPHP UGM. Animals were raised in ambient temperature (25-30°C), fed with pellet and tap water.

### Design of Experiments

There were 5 groups, containing 4 rats in each group. Concentrated extract was suspended in 0.5 % CMC-Na, before being given to the animals orally for 7 consecutive days. Doxorubicin (Kalbe Farma) 4,67 mg/kgBW were administered on intra peritoneal (i.p.) twice a week in day 1<sup>st</sup> and 4<sup>th</sup>, while ETO were given everyday for a weeks.

The first group acts as positive control, being treated with Doxorubicin (Doxo control group). The second group was treated with Doxorubicin and ETO 500 mg/kgBW (treatment group). The third group was treated with Doxorubicin and ETO 1000 mg/kgBW (treatment group). The fourth was treated with ETO 1000 mg/kgBW (ETO control), and the fifth group was controlled without treatment. Each rat blood sampled two times, on day 0 (pre-treatment) and the 8<sup>th</sup> (post-treatment) through the venous ocularis using heparinized capillaries and stored in EDTA vacutainers. Then the number of leukocytes, lymphocytes and neutrophils were determined by hematology analyzer, whereas CD8+ lymphocytes by flowcytometry.

Furthermore, sample preparation is performed 5,0 µl whole blood plus 10µL reagent rat antigen CD3 fluorescein isothiocyanate (FITC)

(Invitrogen) and phycoerythrin (PE) anti-rat CD8+ (eBioscience) and then vortexed and stored in a dark room for 15 minutes. After that, plus 450,0 µl lysing solution (BP FACS) for dilution, vortexed, then set aside in a dark room for 15 minutes. The sample is measured by flowcytometer (FacsCalibur) and analyzed using the program multiset. The data was obtained from relative number of CD8+ T cell lymphocytes.

### Statistical Methods

SPSS/PC 13.0 software was used. Multiple comparisons were done using one-way ANOVA followed by Tuckey Test. The 0.1 level of probability was used as the criterion for significance.

## RESULT

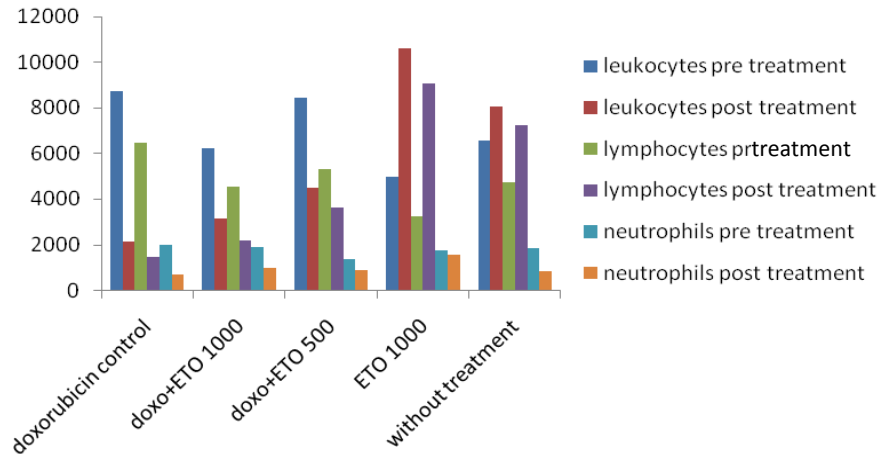
The effects of doxorubicin and/or ETO on the leukocytes, lymphocytes, neutrophils, and CD8+ T cell lymphocytes female Sprague Dawley rats (Figure 1). In general, the profile of hematology analyzer and flowcytometry after treatment in the control doxorubicin group, doxorubicin+ETO 1000, doxorubicin+ETO 500 showed decreasing both the number of leukocytes, lymphocytes, neutrophils, and T cell lymphocytes, CD8+ rather than pre-treatment, whereas the control group ETO 1000 and without treatment showed increasing both the number of leukocytes, lymphocytes, neutrophils, and CD8+ T cell than pretreatment.

Administration of doxorubicin give highest decreasing in the number of immune cells. It's showed that doxorubicin has immunosuppressive effects (Zhang *et al.*, 2005). Treatment of doxorubicin in combination with ETO 1000 mg/kgBB and ETO 500 mg/kgBB increased more the number of immune cells than doxorubicin control group. It showed that the combination of doxorubicin with ETO could reduce the side effects of doxorubicin in suppressing immune system.

The control extract group and the untreated groups of post-treatment showed an increase both the number of leukocytes, lymphocytes, neutrophils, and CD8+ T cells rather than pre-treatment (baseline). Improvement in the control extract groups was higher than the without treatment group showed that the ability to reduce immunosuppressive effects on doxorubicin combined with ETO is caused by the capability of ETO to enhance the immune system (immunostimulatory). Variation of does showed

that RTO 500 m/kgBW decrease the number of immune cells is lower than ETO 1000 m/kgBW. It means that at a dose of 500 mg/kgBW combination of doxorubicin with ETO is more effective in suppressing the side effects of immunosuppressive

doxorubicin. So, the effectiveness in reducing the immunosuppressive effects are non depending dose.



**Figure 1. Hematology Analyzer Profile leukocyte immune cell parameters leukocytes(a), lymphocytes (b), neutrophils (c), flowcitometry profile of CD8 + T cell lymphocytes (d),. In general, the profile of Hematology Analyzer and flowcytometry after treatment in the control group doxo, doxo + ETO 1000, doxo + ETO 500 showed a decrease both the number of leukocytes, lymphocytes, neutrophils, and T cell lymphocytes, CD8 + rather than pre-treatment, whereas the control group ETO 1000 and without treatment showed increasing both the number of leukocytes, lymphocytes, neutrophils.**

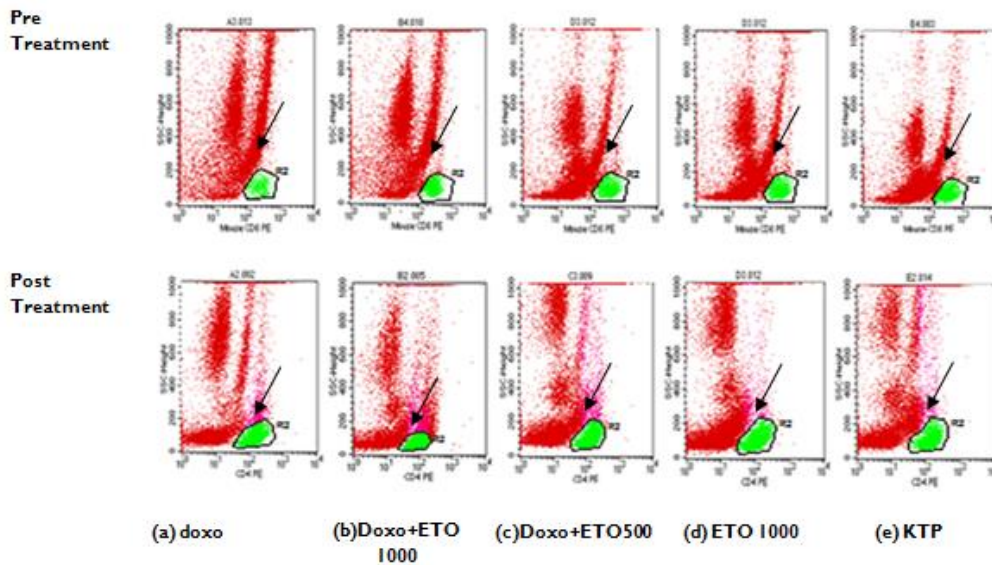
**Table 1. The number of cells leukocytes, lymphocytes , neutrophils**

	Leukosit (/ $\mu$ l)		Limfosit (/ $\mu$ l)		Neutrofil (/ $\mu$ l)	
	pre treatment	post treatment	pre treatment	post treatment	pre treatment	ost treatment
Doxo 4,67 mg/kgBW	8475	2125	6475	1450	2000	675
Doxo+ETO 500 mg/kgBW	6200	3125	4525	2175	1875	950
doxo+ETO 1000 mg/kgBW	8433	4467	5325	3600	1333	867
ETO 1000 mg/kgBW	4975	10600	3225	9075	1750	1525
without treatment	6550	8025	4700	7225	1850	800

This result is supported by flowcytometry profiles of CD8+ T cells lymphocytes relative (Fig. 2 and 3). In general, the fluorescence of lymphocytes CD8+ T cells (green fluorescence) generated in the post-treatment of all groups was smaller than pre-treatment. The fluorescence of doxorubicin smallest in the control group. Group combination of doxorubicin 500 mg/KgBW and 1000 mg/kg, extract control groups and without treatment groups showed higher fluorescence than control doxorubicin group. The green fluorescence indicates the relative number of CD8+ T cell lymphocytes. Then, the relative number of CD8+ T cell lymphocytes time with the total of lymphocytes (Table 1).

mg/KgBW have the increases the number of leukocytes, lymphocytes, neutrophils, cytotoxic CD8+ T cells compared to control doxorubicin groups. But, only doxorubicin combined with ETO 500 mg/KgBW treated group has cytotoxic CD8+ T cells differs significantly from control doxorubicin. Based on these data, we can conclude that administration of ETO is able to increase the number of leukocytes, lymphocytes, neutrophils, the number of CD8+ T cell lymphocytes. The administration can give a single ETO and a combination with doxorubicin showed immunostimulatory activity which also can reduce the side effects of chemotherapy that can be used as a with agent chemotherapy.

Result shows groups of doxorubicin combined with ETO 1000 mg/KgBW and 500



**Figure 2.** Results of data processing flowcytometry CD8 + T cell lymphocytes post-treatment with the multiset. Control doxo group (a); doxo + ETO 1000 (b); doxo + ETO 500 (c); ETO 1000 (d), without treatment (e) on the post treatment has a fluorescence that decreasing from pre-treatment. → green fluorescence indicates the number of CD8 T cell lymphocytes.

**Table 2. The number of CD8+ T limfosit cells**

Group	CD8+ ( $\mu$ l)	
	Pre treatment	Post treatment
Doxorubicin	930	76,6
Doxorubicin+ETO 1000	518	112
Doxorubicin+ETO 500	374	335
ETO 1000	326	776
Without treatment	515	618

## DISCUSSIONS

Doxorubicin is one of the most active cytotoxic agents in current use. The clinical efficacy of the anthracycline, however, has been precluded by immunosuppression (Zhang et al., 2005). Free radical generation and lipid peroxidation have been suggested to be responsible for doxorubicin-induced immunosuppression (Gewirtz, 1999).

In this study prior administration of ETO for twice a week of doxorubicin notably increase the number of the immune cells of female Sprague Dawley rats. Doxorubicin administration induced immunosuppression as manifested by highest decreasing in the number of immune cells.

Being polyphenolic compounds, flavonoids have shown anti-radical and iron-chelating properties in experimental animals (Quiles et al., 2002), and thus would be possible protectors against anthracyclines-induced immunosuppression. *T. officinale* is a source of flavone, luteolin and luteolin-3-o-glucoside (Chun and David, 2005). Hu and Kitts (2005) reported that *T. officinale* flower extract suppresses both reactive oxygen species and nitro oxide and peroxidation lipid in vitro. Our findings showed that combination doxorubicin with ETO can reduce immunosuppression effect of doxorubicin.

This research data can be used as one of the scientific evidence for the development of *Taraxacum officinale* leaves become phytopharmaca and standardized.

## CONCLUSION

*Taraxacum officinale* leaves ethanolic extract 500 mg/kgBW is potentially to develop as immunostimulatory agent for co-chemotherapy with doxorubicin.

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