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Anti-Inflammatory Potential Of Takokak (*Solanum Torvum*) Ethanol Extract In Rats Exposed To 7,12-Dimethylbenz[A]Anthracene (Dmba)

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ABSTRACT

Background: Takokak fruit (*Solanum torvum*) is a type of eggplant containing solasodin, solamargin, solasonin and other phytochemicals components with anti-inflammatory and anti-cancer properties. The purpose of this study was to examine the effects of takokak ethanol extract on TNF- α , IL6, and SOD levels.

Methods: Experimental factorial study design, with the effect of takokak extract concentration factor (0, 400 and 800 mg/kgBW), takokak extract administration time factor including preventive (weeks 1-7) and curative (weeks 6-12) administration, and necropsy factor (necropsy in weeks 12 and 16). The data obtained included rats body weight, TNF- α , IL-6 and SOD levels.

Results: The result showed that the interaction between takokak concentration and handling has significant effect on the increase of TNF- α levels ($p=0.003$) and the decrease of IL-6 levels ($p=0.000$). Interaction between takokak concentration, handling and necropsy has significant effect on the increase of SOD levels ($p=0.010$).

Conclusion: Takokak ethanol extract has significant effect on the increase of TNF- α , and SOD levels, and the decrease of IL-6 levels.

Keywords: Takokak (*Solanum torvum*), inflammation

INTRODUCTION

Carcinogenesis is a multistage process involving molecules and cells with three distinct stages: initiation, promotion and progression. Initiation is a quick and irreversible process with intracellular and extracellular binding. Carcinogen exposure will occur in the early stage, and it will be distributed and transported to the tissues and organs. Metabolism and detoxification will be activated and reactive species will form a covalent bond with the deoxyribonucleic acid (DNA) of the target cells, all of which will cause genetic damage. To the contrary, tumor promotion is a long and reversible process resulting in the activation of proliferation, which is an accumulation of pre-neoplastic cells. Progression is the final stage of neoplastic transformation with invasive and metastatic tumor growth.¹

The relationship between inflammation and cancer has been demonstrated by epidemiology and clinical studies. For example, inflammation is associated with 10 times higher risk of developing colorectal cancer. Anti-inflammation can reduce the incidence of colon cancer. The causes of inflammation are microbial infections, or non-infectious causes such as mechanical and chemical irritation. Chronic inflammation caused by infection may contribute to the carcinogenesis. Inflammation can increase the risk for pharyngeal cancer, pancreatic cancer, and gallbladder cancer.²

Chemical carcinogen such as 7,12-dimethylbenz(a)anthracene (DMBA) will bind to the DNA, which is likely to induce tumor. Several chemopreventive agents can bind to DMBA that may cause tumor, by reducing the binding of DMBA to DNA. Cell proliferation is very important in the regulation of carcinogens that may cause cancer.³

exposure to environmental carcinogens may contribute to cancer risk in humans. Rats are often used as models for human cancer because of their similarities in terms of pathology, cellular authenticity and hormone dependency.⁴ Oxidative stress caused by oxidants can lead to several diseases such as cancer. The high production of ROS (Reactive Oxygen Species) causing oxidative damage to DNA will further contribute to the mutagenesis and carcinogenesis.⁵ Inflammation in the cellular environment may increase mutation rates. Inflammation is activated by ROS and RNS, which can induce DNA damage and gene instability.

Natural ingredients (herbal medicine) had contributed to the development of modern medicine. Herbal medicine from natural ingredients can regulate several inflammatory mediators such as the metabolism of arachidonic acid, peptide, cytokine, and excitatory amino acid, production of second messengers (cGMP, cAMP, protein kinases, and calcium), expression of transcription factors (AP-1, NF- κ B) and proto-oncogene (c-jun, c-fos and c-myc), and expression of proinflammatory molecules such as inducible NO synthase (iNOS), cyclooxygenase (COX-2), cytokine (IL-1, TNF- α), neuropeptide and protease.⁶

A study by Amir and Kumar⁷ showed that crude steroidal glycoalkaloids extract *Solanum ligustrinum* has anti-inflammatory properties in rabbits and guinea pigs. Several components of this substance are scopoletin and B sitosterol-3-o-B-D-glucoside acyl extract from dichloromethane. Another eggplant variant *Solanum nigrum* can be used as anti-inflammation for several diseases such as: liver, spleen, stomach, gut, and uterine diseases, and inflammation of the esophagus and larynx. *Solanum nigrum* alcohol extract can prevent edema in albino rats. The anti-inflammation substance is presumed to be glycoalkaloid steroid and genin steroid.

Several plants known as chemopreventive substances may exert their anti-carcinogenic effect by inhibiting cellular proliferation and inducing cell differentiation and apoptosis. Medicinal herbs are rich in antioxidants as anti-proliferation substances, and may induce apoptosis.⁴ Takokak fruit (*Solanum torvum*) has solasodin, solamargin, solasonin and other phytochemicals components with anti-inflammatory and anti-cancer properties. Based on the previous explanations, a study will be conducted to determine the anti-proliferation and anti-inflammation potential of takokak for cancer cells in animal models. The purpose of this study was to examine the effects of takokak ethanol extract on TNF- α levels, IL6 levels, SOD levels, and cancer cells proliferation.

METHOD

This is an in vivo experimental study with factorial design on rats. The study treatments are takokak extract concentrations of 0 mg/kg BW, 400 mg/kg BW and 800 mg/kg BW. The handlings are preventive takokak extract administration by giving takokak extract in conjunction with DMBA, and curative administration

by giving takokak extract after DMBA. Necropsy will be performed at weeks 12 and 16. The study was conducted in the Laboratory of Physiology Brawijaya University Malang.

Tools and Materials

The materials used are DMBA from sigma and takokak fruits from toga garden of Gunung Leutik village Ciampea Bogor. The rats used are 40 days old female Sprague-Dawley rats, purchased in the Integrated Research and Testing Laboratory (LPPT) Gajah Mada University Yogyakarta. Other chemicals used in this study are anti-human IL-6, primary and secondary IgG antibody, xanthine reagent, xanthine oxidase, TCA, NaThio, mineral mix from the Agricultural Products Technology of Bogor Agricultural University, and 96% ethanol from Makmur Sejati store Malang. The types of fodders used for animal models feeding are corn, soybean, fishmeal, peanut, wheat flour, bone meal, salt, cooking oil and vitamin B complex from Malang local market. The tools used include rotary evaporator, vacuum pump, shaker, oven drying, elisa Hermle Labortechnik GMBH, vortek velp scientifica, shaker-mixer vina instruments and electron microscope.

Research Stages

Takokak Extraction

The takokak fruit was dried at 50°C for 9 days using the oven. Next the takokak powder will be macerated using 96% alcohol 1:7 for 24 hours. The extract fluid was evaporated until a thick extract remains. Afterwards the extract is stored at low temperatures until used in the following studies.

Fodder making

Rats fodder is made from corn, roasted fish, peanut roaster, soybean roaster, roasted bone, wheat flour, mineral mix and vitamin B complex, each materials was finely ground.⁸ The composition of rats fodder consisting of; cornstarch (70%), fish meal (5%), bone meal (1,5%), soybean flour (10%), peanut flour (5%), mineral mix (0.2%), vitamin B complex (1 tablet/kg), cooking oil (1%) and salt (0.2%). The fodder was mixed and made every 10 days.

Animal Models

Each rat was placed in a plastic box with husks covered floor, with room air humidity of about 50-60% and room temperature of $\pm 25^\circ\text{C}$, standard ration was given to all white rats during adaptation and treatment period. During adaptation period the rats were given standard ration and drinks *ad libitum*. Rats' body weights were observed at week 0, 1, 2, and 3 and so forth up to week 16. Fodder consumption was observed everyday by weighing the residual fodder. Minimum number of rats for each treatment is two, which was calculated from the formula $((t-1)(r-1)) > 15$. This study

had been approved by the Research Ethic Committee of the Polytechnic of Health Malang Reg. no. 163/2012.

Diet and Treatments for The Animal Models

A total of 36 rats were divided into 6 groups, each groups consisting of 6 rats. The rats were adapted for 10 days. Afterwards the rationing was varied based on the treatment type. DMBA was given 20 mg/kg/BW orally 2 times per week for 5 weeks in 0,5 ml corn oil⁹. Rat necropsy will be performed on weeks 12 and 16, 3 rats each, and then the TNF- levels, IL-6 levels and SOD levels were analyzed. Anesthesia was performed by ketamil injection 0.1 ml/rat (10 mg in 50 ml) or 0.02 mg/rat. About 2.5 ml of blood was taken from the heart using 5 ml syringe.

The treatment was given as follows:

1. Group I, DMBA (positive control)
2. Group II, DMBA and takokak extract 400 mg/kgBW 1x/day from weeks 1-7.
3. Group III, DMBA and takokak extract 800 mg/kgBW 1x/day from weeks 1-7.
4. Group IV, DMBA (positive control)
5. Group V, DMBA and takokak extract 400 mg/kgBW 1x/day from weeks 6-12.
6. Group VI, DMBA and takokak extract 800 mg/kgBW 1x/day from weeks 6-12.

TNF- Levels

Coating antigen is washed in micro tube plate by mixing serum with 50 µl coating buffer at a 1:20 ratio, and incubated for 1 night at 4°C. Then it was washed with 50 µl 0.2% PBS tween 3 times for 3 minutes each. Next blocking buffer (1% BSA in PBS) was added. Then it was incubated at room temperature for 30 minutes. Subsequently it was washed with 50 µl 0.2% PBS tween 3 times for 3 minutes each. Primary antibody coating was added using primary antibody and PBS at a 1:500 ratio, and then incubated for 2 hours at room temperature. Then it was washed with 50 µl 0.2% PBS tween 3 times for 3 minutes each. Secondary antibody coating was added using conjugated AP (Alkaline Phosphatase) IgG antibody and PBS at a 1:1000 ratio, and then incubated for 1 hour at room temperature. Subsequently it was washed with 50 µl 0.2% PBS tween 3 times for 3 minutes each. SA-HRP at a 1:1000 ratio was added and then incubated for 1 hour at room temperature, and washed using 50 µl 0.2% PBS tween 3 times for 3 minutes each. Sure blue TMB (tetramethylbenzidine) was added, and then incubated for 30 minutes at room temperature or in the dark. The reaction was terminated by adding 1N HCl, and then it was incubated for 15 minutes. Subsequently it was analyzed using elisa at a 450 nm wavelength.¹⁰

IL-6 Levels

Coating buffer at a 1:4000 ratio was added to the serum, and allowed to stand overnight at 4°C. It was

then washed using PBS tween for 6 times. Pure feel anti-human IL-6 diluted in assay buffer at a 1:4000 ratio was added. Subsequently it was incubated at room temperature for 2 hours while shaken. It was then washed using PBS tween for 6 times and 100 µl biotin gout anti-human IgG at 1:800 ratio in assay buffer was added. Subsequently it was incubated at room temperature for 60 minutes while shaken. It was then washed using PBS tween for 6 times and 100 µl SAHRP at 1:500 ratio in 0.5 µl/2ml assay buffer was added. Next it was washed using PBS tween for 6 times and 100 µl TMB substrate was added. Subsequently it was incubated at room temperature for 10 minutes while shaken, and 100 µl stop solution was added, then analyzed using elisa at 450 nm wavelength.¹¹

SOD Levels

Blood was centrifuged at 3000 rpm for 10 minutes and the serum was collected. Serum was centrifuged at 6000 rpm for 10 minutes, and the supernatant was collected. Sample volumes of 100 µl were taken and 100 µl of xanthine, xanthine oxidase, NBT, PBS reagent were added respectively, next PBS was added to the final volume of 1000 µl, incubated at 30°C for 30 minutes, and then analyzed using elisa at 580 nm wavelength.¹²

Data analysis

Data was presented using descriptive statistics and analysis of variance. The study had been approved by the Research Ethic Committee of the Polytechnic of Health Malang.

RESULT

Body Weight

The range of body weight for the rats in this study is between 122.5 g to 256.0 g. The mean body weight of rats is 195.7 g. The mean body weight is 175.6 g for preventive treatment (during takokak administration) and 163.3 g for curative treatment.

Analysis of variance showed that the interaction between takokak concentration and increased concentration and preventive or curative treatment have a significant effect on body weight ($p=0.022$), increased takokak concentration and preventive or curative treatments were found to play a role in weight gain. Body weight of rats from preventive group is presented in Figure 1 and body weight of rats from curative group is presented in Figure 2.

TNF- Levels

The range of TNF- levels in this study is between 23.50±2.29 pg/ml to 68.00±13.44 pg/ml. The mean TNF- level is 46.90±21.9 pg/ml. The mean TNF- level is 39.43±5.50pg/ml for preventive treatment and 50.19±7.0 pg/ml for curative treatment. The mean TNF- level is 42.83±3.25 pg/ml for necropsy at week 12 and 46.79±9.33 pg/ml at week 16. TNF- levels are presented on Table 1.

Analysis of variance showed that the takokak concentration has significant effect on TNF- levels ($p=0.000$), higher takokak concentration is correlated with greater increase in TNF- levels. TNF- level for preventive treatment is lower than curative treatment. Analysis of variance showed that preventive and curative treatments have significant effect on TNF- levels ($p=0.021$). TNF- level for necropsy at week 12 (42.83 ± 3.25 pg/ml) showed lower propensity than TNF- level for necropsy at week 16 (46.79 ± 9.3 pg/ml), but the difference is not statistically significant ($p=0.882$). Time of necropsy at weeks 12 and 16 has no effect on TNF- levels.

Interaction between takokak concentration with increased concentration and preventive or curative treatment has significant effect on TNF- levels ($p=0.011$), increased takokak concentration and preventive or curative treatment play a role in the increase of TNF- levels.

IL-6 Levels

The range of IL-6 levels is between 9.34 ± 0.1 pg/ml to 21.81 ± 0.40 pg/ml. The mean IL-6 level is 16.62 ± 6.3 pg/ml. The mean IL-6 level is 16.82 ± 6.4 pg/ml for preventive treatment and 16.43 ± 6.2 pg/ml for curative treatment. The mean IL-6 level is 16.50 ± 6.2 pg/ml for necropsy at week 12 and 16.56 ± 7.5 pg/ml at week 16. The IL-6 levels are presented on Table 2.

Analysis of variance showed that different takokak concentration has significant effect on IL-6 levels ($p=0.000$), higher takokak concentration is correlated with lower IL-6 levels. Takokak is assumed to have such effect because it contains alkaloid, triterpenoid and tannin that can counteract free radicals and thus suppressing inflammation. Preventive and curative treatment has no effect on IL-6 levels ($p=0.265$), preventive and curative treatments did not cause a difference in IL-6 levels. Time of necropsy (weeks 12 and 16) has no effect on IL-6 levels ($p=0.904$). Interaction between takokak concentration with different concentration and preventive or curative treatments has significant effect on IL-6 levels ($p=0.000$), higher takokak concentration and preventive or curative treatment play a role in the decrease of IL-6 levels.

Superoxide Dismutase (SOD) Levels

The range of SOD level is between 4.37 ± 0.20 μ /ml to 7.96 ± 1.10 μ /ml. The mean overall SOD level is 5.93 ± 2.30 μ /ml. The mean SOD level is 5.95 ± 2.3 μ /ml for preventive treatment and 5.90 ± 2.2 μ /ml for curative treatment. The mean SOD level is 6.55 ± 2.3 μ /ml necropsy at week 12 and 5.31 ± 1.9 μ /ml at week 16.

Analysis of variance showed that takokak concentration has significant effect on SOD levels ($p=0.023$), higher takokak concentration is correlated with higher SOD levels. Preventive and curative treatments have no effect on SOD levels ($p=0.480$), preventive treatment showed higher SOD levels than

curative treatment. Time of necropsy (weeks 12 and 16) has significant effect on SOD levels ($p=0.000$), necropsy at week 12 showed higher SOD levels than necropsy at week 16.

Interactions between takokak concentration and preventive or curative treatment have significant effect on SOD levels ($p=0.012$), concentration of takokak given as preventive or curative treatments play a role in the increase of SOD levels. Interaction between takokak concentration, preventive or curative treatments and time of necropsy (weeks 12 and 16) have significant effect on SOD levels ($p=0.010$), higher takokak concentration and preventive or curative treatment and time of necropsy at week 12 and 16 play a role in the increase of SOD levels. The SOD levels are presented on Table 3.

DISCUSSION

Body Weight

The fodder consumed by rats has caused a significant increase in body weight every week, this suggests that the fodder consumed was adequate because it can cause weight gain. The administration of takokak with higher concentration as preventive or curative treatments can cause weight gain. However, weight gain for preventive treatment is higher than curative treatment. Preventive treatment group showed propensity to have more weight gain for higher takokak concentration, whereas the curative group showed propensity to have lower body weight for higher takokak concentration. This might be explained by less number of rats with cancer in the preventive treatment group, and more rats with cancer in the curative treatment group. Cancer might influence the appetite, thus rats on curative treatment group have a tendency to show a decrease in body weight.

TNF-

Cytokine is a substance that can facilitate cancer growth, invasion and metastasis by causing DNA damage and inhibiting DNA repair through RO (reactive oxygen), and also by deactivating tumor suppressor gene.¹³ TNF- is a cytokine that plays a role in inflammation, immunity, cellular homeostasis and tumor progression.¹⁴ Takokak extract can increase TNF-

levels. Takokak is assumed to have such effect because the extract contains certain phytochemicals components such as alkaloid, tannin, triterpenoid and solasodin and solamargin, which have the ability to increase pro-apoptotic protein or inhibit anti-apoptotic protein that may reduce inflammation. Higher takokak extract administration is correlated with more cancer cell death thus reducing the inflammation. The result of the current study is different with the research from Hanaa *et al.*,¹⁵ where red sea soft coral (mixture of *Saecophyton trocheliophorum*, *Sinularia polydactyla*, *Xenia macrospiculata*) methanol extract can decrease the TNF- levels on rats fed with DMBA (10 μ g/rat in

0.2 ml acetone) and 10 µg TPA (Topical Tumor Promoter) in 0.2 ml acetone intraperitoneal.

IL-6 Levels

IL-6 is a pro-inflammatory cytokine produced by T cells and macrophages to stimulate immune response during infection or tissue damage.¹⁶ Based on a study by Rinco, IL-6 also plays a role in several diseases such as cancer, neurological disease and metabolic syndrome. The increase of IL-6 level in tumor cells is correlated with cancer growth. IL-6 is also a target for cancer therapy.¹⁷ Up-regulated cytokine (IL-6) levels in inflammation can have an impact on cancer cells. Several cancers with metastasis showed an increase in IL-6 levels. Some cancer cells can stimulate the surrounding stromal cells (connective tissue) to produce a number of cytokines.¹⁸ IL-6 blocking may be of benefit for several diseases.¹⁹ The results of this study showed that takokak extract administration may significantly reduce IL-6 levels, presumably because takokak extract contains several phytochemicals that can counteract free radicals. Reduction in radical compounds will cause the body reaction to produce less IL-6. Preventive takokak extract administration group (takokak extract was administered together with DMBA) showed lower IL-6 levels compared to curative group (takokak extract was administered after DMBA) during necropsy at week 12, and this finding showed that preventive takokak extract administration may improve IL-6 levels. On the contrary, preventive takokak extract administration during necropsy at week 16 did not improve IL-6 levels, possibly because the rats already had more advance inflammation during necropsy at week 16, hence takokak extract cannot suppress inflammation. This results showed similarities with Alkaladi²⁰ where Silymarin administration (flavonoid extracted from *Silybum marianum*) can decrease the levels of IL-6.

IL-6 also plays a role in several diseases such as cancer, neurological disease and metabolic syndrome. The results showed that chronic inflammation may lead to an increase in cancer risk, promoting tumor progression, and promoting metastasis. In the early stage of tumor progression, inflammatory mediators such as cytokine, ROS and RNS is derivative of tumor-infiltrating immune cells.²¹

SOD Levels

Superoxide dismutase (SOD) is an enzyme (co-factor protein) that catalyzes the reaction with superoxide (O_2^-) into oxygen and hydrogen peroxide. Intracellular SOD is the first substance to fight ROS

(reactive oxygen species).²² Antioxidant works through 3 different mechanism: 1). Prevention. Preventive antioxidants attempt to stop the formation of ROS. These include superoxide dismutase (SOD) that catalyzes the dismutation of superoxide (O_2^-) to H_2O_2 and catalase that breaks it down to H_2O ; 2). Interception. Interception of free radicals is mainly by radical scavenging of peroxy radicals, such as vitamins C and E, glutathione, flavonoids, polyphenol, etc.; 3). Repair and reconstitution, mainly by influencing enzymes involved in free radicals.²³ SOD is an antioxidant enzyme with the ability to suppress proliferation and have an effect on inflammation. Inflammation-induced cancer can be explained by the activation of immune cells and cytokine by oxidative stress from free radicals.²⁴

Takokak extract administration can increase the SOD levels. Preventive and curative administration of takokak extract and time of necropsy at weeks 12 and 16 is correlated with the increase of SOD levels. This effect is attained because takokak extract contains phenol (antioxidant) which known to be able to stop ROS production, counteract free radicals and influence several enzymes related to free radicals. Increased SOD level, an antioxidant enzyme, may counteract free radical compounds, and with the decrease in free radical compounds, the body will produce less pro-inflammatory protein such as IL-6. This results were in line with Paliwal,²⁵ in which the administration of 200-400 mg/kgBW *Moringa oleifera* (moringa leaves) ethanol-water extract was able to increase the SOD levels on mice fed with 15 mg/kgBW DMBA for 15 days.

CONCLUSIONS

Takokak concentration has significant effect on the increase of TNF- and SOD levels, and the decrease of IL-6 levels.

RECOMMENDATION

Takokak extract at a dose of 800 mg/kgBW can be used to prevent inflammation.

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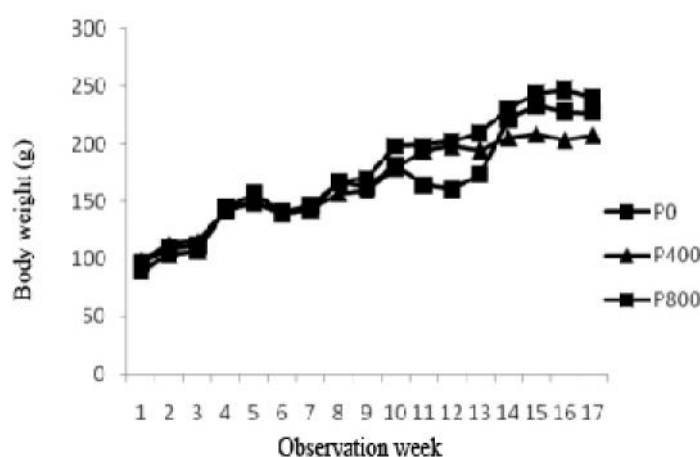


Figure 1. Rats body weight in the preventive group.

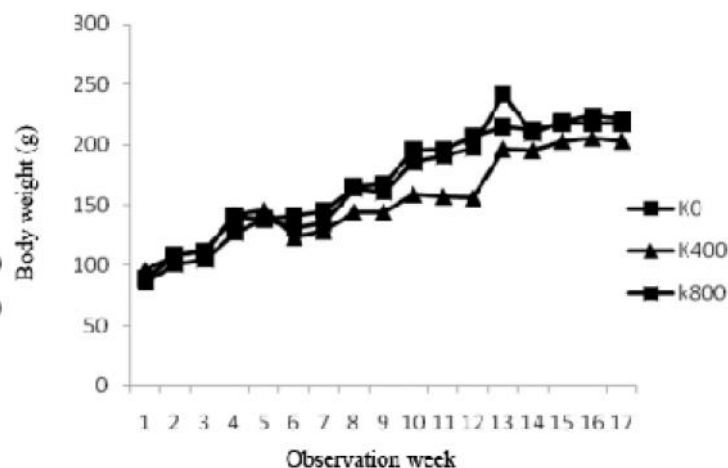


Figure 2. Rats body weight in the curative group.

Table 1. TNF- levels of the rats during the study

Extract concentration mg/kg	Time of necropsy Week				Mean		Mean
	12		16		Preventive	Curative	
	Preventive	Curative	Preventive	Curative			(pg/ml)
0	26,50±0,0	28,33 ±1,6	23,50±2,2	28,67±3,0	24,70±10 ^C	28,50±9,1 ^{CB}	26,3±10,5 ^c
400	30,00 ±0,7	62,33±6,6	44,25±11,6	4,00±9,1	37,12±22 ^B	59,00±20 ^A	49,3±22,2 ^b
800	44,33±5,2	65,50±5,2	68,00±13,4	62,33±16,3	66,50±24 ^A	63,91±23 ^A	65,1±25,2 ^a
Mean	33,61±2,0	52,06±4,5	45,25±9,1	48,33±9,5	39,43±5,5 ^b	50,19±7,0 ^a	46,9±21,9
	Kt			0,000*			
v a l u e	Pn			0,021*			
	N			0,882			
	Kt*pn			0,011*			
	Kt*N			0,827			
	Pn*N			0,165			
	Kt*pn*N			0,216			

Description:

Kt: Takokak concentration

Pn: Handling

N: Necropsy

Kt*pn : Interaction between takokak concentration and handling

*: significant

Uppercase notation showed interaction

Table 2. IL-6 levels of the rats during the study

Extract Concentration mg/kg	Time of necropsy Week				Mean		Mean
	12 Preventive	Curative	16 Preventive	Curative	Preventive	Curative	
	(pg/ml)						
400	16,71±0,5	18,71±0,8	17,14±0,5	19,57±1,4	16,96±6,5 ^B	19,26±6,5 ^A	18,11±6,6 ^a
800	20,21±0,0	15,07±0,7	18,01±3,8	14,21±0,1	19,10±7,3 ^A	14,72±6,1 ^C	16,91±6,7 ^b
Mean	9,34±0,1	14,71±0,4	21,81±0,4	15,91±0,1	14,38±5,3 ^C	15,28±6,0 ^C	14,83±5,7 ^c
p	Kt			0,000*			
v	Pn			0,265			
a	N			0,904			
l	Kt*pn			0,000*			
u	Kt*N			0,052			
e	Pn*N			0,265			
	Kt*pn*N			0,899			

Kt: Takokak concentration

Pn: Handling

N: Necropsy

Kt*pn : Interaction between takokak concentration and handling

*: significant

Table 3. SOD levels of the rats during the study

Extract concentration mg/kg	Time of Necropsy Week				Mean		Mean
	12 Preventive	Curative	16 Preventive	Curative	Preventive	Curative	
	(µ/ml)						
0	7,96±1,1 ^A	6,07±0,4 ^{CD}	5,34±0,3 ^{CDE}	4,90±0,3 ^{DE}	6,38±2,4 ^{AB}	5,48±1,9 ^B	5,89±2,3 ^{ab}
400	5,23±1,2 ^{CDE}	6,42±0,8 ^{BC}	5,66±0,5 ^{CDE}	4,37±0,2 ^E	5,48±2,0 ^B	5,60±2,1 ^B	5,54±2,1 ^b
800	6,33±0,1 ^{BC}	7,46±1,1 ^{AB}	5,51±0,1 ^{CDE}	5,79±0,3 ^{CD}	6,00±2,5 ^{AB}	5,62±2,5 ^A	6,34±2,5 ^a
Mean	6,50±0,8	6,65±0,8	5,50±0,3	5,02±0,3	5,95±2,3 ^a	5,92±2,2 ^a	5,93±2,3
p	Kt			0,023*			
v	Pn			0,480			
a	N			0,000*			
l	Kt*pn			0,012*			
u	Kt*N			0,197			
e	Pn*N			0,199			
	Kt*pn*N			0,010*			

Kt: Takokak concentration

Pn: Handling

N: Necropsy

Kt*pn : Interaction between takokak concentration and handling

*: significant

Synopsis:

Takokak (*Solanum torvum*) ethanol extract plays a role in reducing inflammation, have significant effect in reducing the IL-6 levels and increasing the TNF- and SOD levels.

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