

## The Effect of $\text{KIO}_3$ and KI Salt Towards Iodine Levels ( $\text{I}_2$ ) in Urine, Malondialdehyde (MDA) and The Histology of Thyroid Gland of Goitrogenic Rat

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### ABSTRACT

Goitrogenic substances can inhibit of iodine taking by the thyroid gland. Thus iodine concentration in thyroid gland will be low, and this phenomena is indicated by inflammation in the thyroid gland. Moreover, it can cause releasing of an excessive amount of free radicals. This radicals, in the body, causes oxidative stress and also increase the levels of malondialdehyde (MDA). This is also as an indicator for lipid peroxidation and the decreasing of urinary iodine excretion levels (EIU). The treatment with  $\text{KIO}_3$  and KI salt was intended to study the level of supplementation of iodine ( $\text{I}_2$ ) toward level of MDA in serum and histological description of rat's thyroid gland. The MDA levels was determined through TBA test (Thio Barbituric Acid), meanwhile the histological pattern of rat thyroid gland was determined by Hematoxylen-Eosin staining (HE). The results indicated both of  $\text{KIO}_3$  and KI salt significantly ( $p < 0.01$ ) reduced MDA level in the serum. Treatment with  $\text{KIO}_3$  salt gave 33.62% while KI salt slightly higher (37.02%). In addition, both of treatments displayed an recovering effect in thyroid gland.

Key word: Goitrogenic, Iodine, Malondialdehyde,  $\text{KIO}_3$  and KI salt therapy.

### INTRODUCTION

Recent National Survey in Indonesia (1998) reported 53.8 million people live with iodine deficiency risk, 20 million suffer from goitre, 290 thousand estimated to suffer from cretinism, and approximately 9 thousand infants born each year [1]. Thyroid disease in American country continues growth, and approximately 17,000 cases occurred each year, and about 1,700 of them resulting death. A study in England from Whickham Study of the United Kingdom reported that goitre is suffered in 16% from the total population by people. In the Framingham study reported that the ultrasound examination of thyroid nodules found in older men over 60 years at 3%, while for 48-year-old woman recorded at 36% [2]. In America, majority cases of thyroid goitre caused by autoimmune and known as Hashimoto's disease. But, mostly the cause of goitre is due to iodine deficiency. It is estimated that about 200 million goitre among the 800 million people suffering from iodine deficiency. Whickham study reported the prevalence of goiter 26% of women, compared to only 7% of men or women genders dominated by a ratio of 4: 1 [2].

A variegated goitrogenic can cause a disease in inflammation. While inflammation of the membrane in the gland thyroid (adipose) can be caused by cytokine pro-inflammation and high production of free radicals. This result is the onset of oxidative stress in which the

imbalance of radicals level in the body and lead to damaging of the cell membrane. This process can be identified by increasing of malondialdehyd (MDA) level and other indicator for lipid peroxidation. Injecting or consuming of the antioxidant substances from outside the body can suppress elevation of MDA level in the body. Generally, treatment of iodine can affect by improving the level of iodine deficiency in the body. But, prolonged the iodine deficiency will interfere the process in formation of thyroid hormones. When the body has iodine deficiency, the concentration of thyroxine hormone in the blood is low. This condition stimulates the body to enhance the thyroid tissue. As a result is enlargement of thyroid gland, or is called a goiter [3]. Theoretically, therapy using substance contained iodine such as  $\text{KIO}_3$  and KI salt can increase iodine level, and this can be monitorred through urine iodine status, the iodine content in urine, lowering levels of malondialdehyde (MDA).

## EXPERIMENT

### Animal treatment

All conditions of experiment and handling of the animals were conducted following the protocols approved by Ethical Clearances Committe in Brawijaya University (238-KEP-UB). A number of 20 rats (*Rattus norvegicus*) (female, body weight 125-200 g) were housed at room temperature in the animal house. This was gently care in the Laboratory of Cellular and Molecular Biology, Mathematics and Sciences Faculty, Brawijaya University Malang and were exposed to alternate cycles for 12 h light and dark. The rats were divided into four group, a healthy group, goitrogenic group, goitrogenic group treated with  $\text{KIO}_3$  salt and goitrogenic group treated with KI salt. The goitrogenic rats groups were sonde injected with 1.75 mL KSCN in mouth and incubated for 3, 6, 9, 12, and 15 days. Then after 7 days, the goitrogenic rats group for divided into group treated with the  $\text{KIO}_3$  and the group treated with KI salt. A dose level given oral was 80 mg/Kg of body weight (BW) for 14 days. After day 15, group of rats were dissected. Rats were killed by neck dislocation, and rat serum, urine and gland thyroid were taken. The serums were taken in abdomen section, heart. This was put into pakutener non-EDTA and left for three hours and centrifuged at 600 rpm for 15 min. This serum was further analyzed. Moreover, gland thyroid were also taken and the skin were slashed, and washed with 0.1% NaCl and immersed in 4% PFA for seven days.

### Analysis of iodium level using Cerric Ammonium Sulfat (CAS) test

Urine samples 250  $\mu\text{g}$  was pipetted and shaken up in a sealed test tube. Each of tube contain sample were added 0.75 mL of iodine. Ammonium persulfate 1.0 mL was also added. After that, all the sample tubes were heated in 91-98  $^{\circ}\text{C}$  for 60 min. The tubes were cooled down to room temperature, and 3.5 mL of arsenite ( $\text{As}_2\text{O}_3$ ) was added followed by homogenization for 15 min. Then, 4.0 mL cerric ammonium sulfate was dropped in each tube and mixed for 15 to 30 second. Then, each tubes was analysed using UV-Vis spectrophotometer at 420 nm with interval time 30 second.

### Analysis MDA levels using TBA test

The rats serum 100  $\mu\text{l}$  in tube was added 550  $\mu\text{l}$  aquadest, 100  $\mu\text{l}$  TCA 100%, 250  $\mu\text{l}$  HCl 1 N, and 100  $\mu\text{l}$  Na-thio 1%. This mixture was homogenized with a vortex, centrifuged at 550 rpm for 15 min, and the supernatant was taken. The resulted solution was incubated in water bath at 100 $^{\circ}$  C for 20 min, and left to room temperature and measured using UV-Vis spectrophotometer at 532 nm.

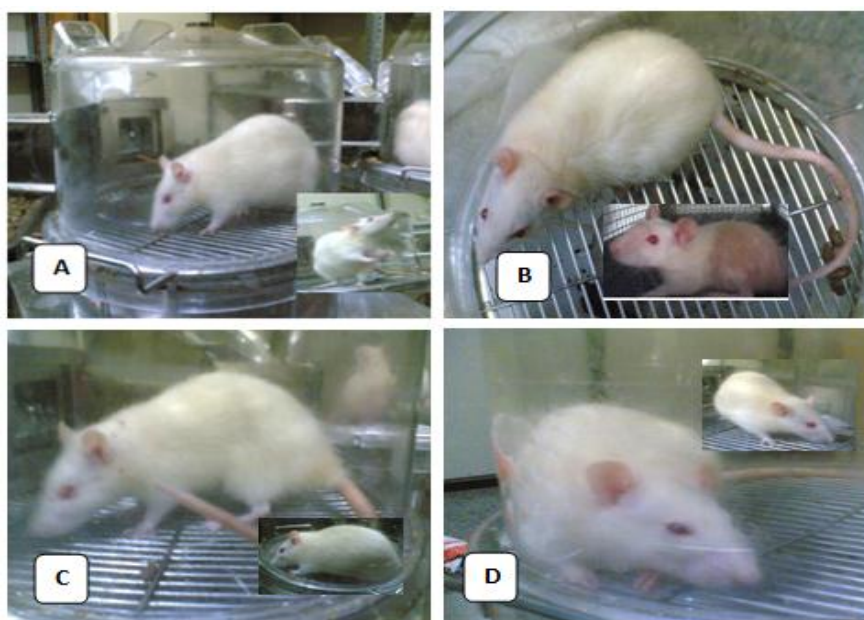
### Histological analysis using Hematoxylen-Eosin (HE) staining method

A prepareate of the rats thyroid gland was immersed in 1-3 xylol for 5 min, and was put into the various concentration of ethanol, from absolute ethanol 1-3, ethanol 95%, 80%, and 70% respectively for 5 min. This was soaked in aquadest for 5 min and put into hemotoxylen dyes for  $\pm$  10 min. Then, it was washed over flowing water for 30 min, and rinsed with aquadest before continued to coloring with eosin dye. Then, it was inserted to eosin alcohol for 5 min and soaked in the aquadest to release the excess of eosin. Moreover, in the dehydration process, the equipment was inserted in the graded ethanol 80%, 90%, and 95% to the 1-3 absolute of ethanol. Then, in the clearing process, it was completed by placing in the xylol 1, 2 and was further dried. The result prepareate was mounted using entellan, and the dried and stained ultrathin sections were observed using a microscope (Olympus BX53) with a magnification of 100 times.

## RESULTS AND DISCUSSION

### Effect thiocyanate (KSCN) and level of iodium in urine

Direct observation from physical appearance of the treated rats indicated changing in the physiological bodies of rat suffering from goitre due to exposure to thiocyanate (KSCN). KSCN experimentally inhibits transportation of iodide ions in the body. Then, it will lower iodine content. Physical phenomena can be see also from mouse.



**Figure 1.** Normal rat (Healthy, A), rats exposed to KSCN (Goitrogenic, B), salt therapy rats with  $\text{KIO}_3$  (Therapy, C), and salt therapy KI rats (Therapy, D).

Little appearance changing was observed on treated rats (**Figure 1**). The changing was compared to healthy rat (A) as negative control, rat exposed with thiocyanate or rat with goitrogenic problem (B), goitrogenic rat got tretament with  $\text{KIO}_3$  (C), and goitrogenic rat got treatment with KI (D). Summary of the diagnosis was presented in **Table 1**.

**Table 1.** Observation result on physiological alteration during observation on groups of treated rats

Indicator*	Physical observation			
	Control Group	KSCN Group	KSCN +KIO <sub>3</sub> Group	KSCN +KI Group
Eye appearance	1	2	2	2
Feather appearance	1	3	2	2
Eating habit	1	1	1	1
Body sighting	1	4	3	3
Rat behavior	1	3	2	2

\*Note: Eye color: 1. Bright-red; 2. Not Bright-red; Feather: 1. Intact and clean; 2. Intact and dirty; 3. Fall out and dirty; Eating Habits: 1. a lot; 2. Medium; 3. Slightly; Behavior: 1. Normal; 2. Aggressive; 3. Idle and lethargic; Rat Behaviour: 1. Length and lightweight; 2. Length and weight; 3rd. Short and light; 4. Short and heavy

The affect of treatment with KIO<sub>3</sub> and KI salt toward quantity measured of iodine level on rat urine was summarised on **Table 2**. Iodine deficiency was observed on group of rats administered with thiocyanate. This was a goitrogenic rats or sick rats, and was applied as positive control. The iodine level on urine was recorded on  $0.035 \pm 0.022$  ppm. This was lower level than that in healthy rat (negative control rat). It has iodine level on  $0.180 \pm 0.011$  ppm. In addition, treatment of the goitrogenic rats with KIO<sub>3</sub> salt slightly improved the iodine level on urine to  $0.063 \pm 0.008$  ppm than that in sick rats. Indeed, for group rats treated with KI salt gave better improvement of iodine content. It reached to  $0.081 \pm 0.007$  ppm. However, both therapy using KIO<sub>3</sub> and KI salt still lower than iodine content on healthy rats ( $0.180 \pm 0.011$  ppm). In other word, for the treatment goitrogenic rat with KIO<sub>3</sub> and KI salt, there were an lowering level of iodine level on urine compared to the healthy rat or the were improvement of iodine level of goitrogenic rats after treatment with KIO<sub>3</sub> and KI salt. This result was also analysed using statistical method with significant level ( $p < 0.01$ ), and it gave significant difference above 99% (**Figure 2**).

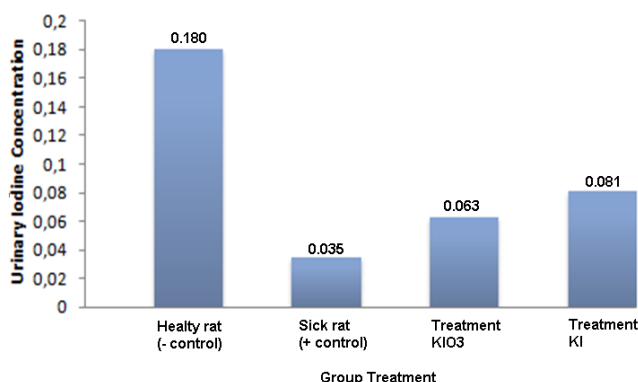
**Table 2.** Urine Iodine Levels

Group of rats	Levels of iodine average (ppm)	Iodine lowering levels towards negative control (%)	Iodine increasing levels towards positive control (%)
Healthy (negative control)	$0.180 \pm 0.022$	-	-
Sick (positive control)	$0.035 \pm 0.011$	80.55	-
Treatment with KIO <sub>3</sub>	$0.063 \pm 0.008$	65.00	44.44
Treatment with KI	$0.081 \pm 0.007$	55.00	56.79

It was also common stated that the incidence of iodine deficiency can be caused by potassium thiocyanate (KSCN) which inhibit iodine uptake by thyroid gland to bind organic iodine such as moniodothyrosine and diiodothyrosine. Both are part of the triglobulin located in each cell. These cause severe competition between serum thiocyanate and thyroxine to bind it, as a result of an increasing in serum thyroxine [4]. Salt administration using KIO<sub>3</sub> and KI in mice on groups of goitrogenic rats reduces the level of iodine transport barriers. The bounded iodine in KIO<sub>3</sub> and KI salts are converted to iodide (I<sup>-</sup>) during

hormogenesis, process in thyroid hormone. This can increase iodine, even though cause inhibition of peroxide generation due to an increasing of I-intra-thyroidal content by supplementation of  $\text{KIO}_3$  and KI salt. Treatment with  $\text{KIO}_3$  and KI can also affect pituitary to work harder secreting TSH and stimulate the thyroid gland to producing more thyroid hormone. As a result initiation of thyroid hyperplasia and hypertrophy (goiter).

**Figure 2.** Comparison of the iodine level in urine of healthy rats (*Rattus norvegicus*), goitrogenic rats, and therapies rat with  $\text{KIO}_3$  and KI salt



### Levels of malondialdehyde (MDA) in rats serum

Measurement of malondialdehyde concentration contained in serum of the treated groups of rat was tabulated in Table 3 and displayed as picture on Figure 3. It was recorded that treatment with injection of  $\text{KIO}_3$  and KI salt in goitrogenic rat reduced the MDA concentration on serum. As a negative control, group of healthy rats have malondialdehyde level  $2.820 \pm 0.014$  ppm. It is normal MDA level on serum. However, for group of rat with goitrogenic disease has three fold much higher of MDA level. It was measured on  $7.857 \pm 0.041$  ppm. Treatment with  $\text{KIO}_3$  and KI salt on groups of goitrogenic rats indicated reduction of their MDA concentration. Treatment with  $\text{KIO}_3$  gave a slight reduction to  $5.215 \pm 0.146$  ppm while treatment with KI salt gave much lower value. It was reached to  $4.925 \pm 0.095$  ppm. Even though this concentration indicated reduction, however this concentration still much higher than that for group of healthy rats ( $2.820 \pm 0.014$  ppm).

**Table 3.** Malondialdehyde levels (MDA) in Rat Serum

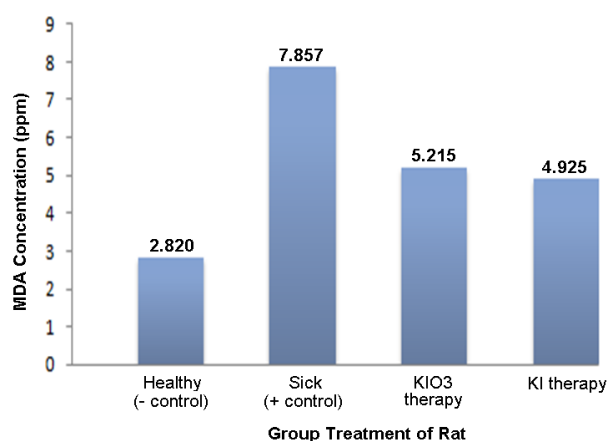
Group of rats	Levels of MDA in average (ppm)	Iodine increasing level toward control (%)	Iodine lowering level toward control (%)
Healthy (negative control)	$2.820 \pm 0.014$	-	64.10
Sick (positive control)	$7.857 \pm 0.041$	178.61	-
Treatment $\text{KIO}_3$	$5.215 \pm 0.146$	84.920	33.62
Treatment KI	$4.925 \pm 0.095$	74.640	37.02

In other word, the MDA levels of rat serum (*Rattus norvegicus*) decreased after treatment with  $\text{KIO}_3$  salt by 33.62% while treatment with KI reached to 37.02%. This decreasing level was caused by iodine content in  $\text{KIO}_3$  and KI salt. This iodine activate the process of inhibition for transporting goitrogen thiocyanate (KSCN). Improvement number iodine in thyroid gland may also accelerate the activity of iodine in stimulating the secretion of thyroid hormone, triiodothyroxine ( $\text{T}_3$ ) and thyroxine ( $\text{T}_4$ ). This will increase TSH level in thyroid gland, and finally will reduce a tissue destruction in thyroid gland.



Moreover, reducing MDA level means inhibit the free radical substances. This inhibition mechanism correlate to process in lipid peroxidation by both salts of  $\text{KIO}_3$  and KI salt as antioxidant agent which counter act the free radicals in body. Iodine content in  $\text{KIO}_3$  and KI salt possibly was the resposible substance as antioxidant [5]. Malondialdehyde (MDA) which is the product of peroxidation as an indicator that a lipid disorder was occuring. Therefore, the high concentration of malondialdehyde in groups of goitrogenic rat serum was also an indication of the high levels of the adipose membrane tissue disorder.

**Figure 3.** Comparison of the value of Serum levels of MDA in healthy rats (*Rattus novergicuss*), goitrogenic rats, and therapies rat with  $\text{KIO}_3$  salt and therapies rat with KI salt

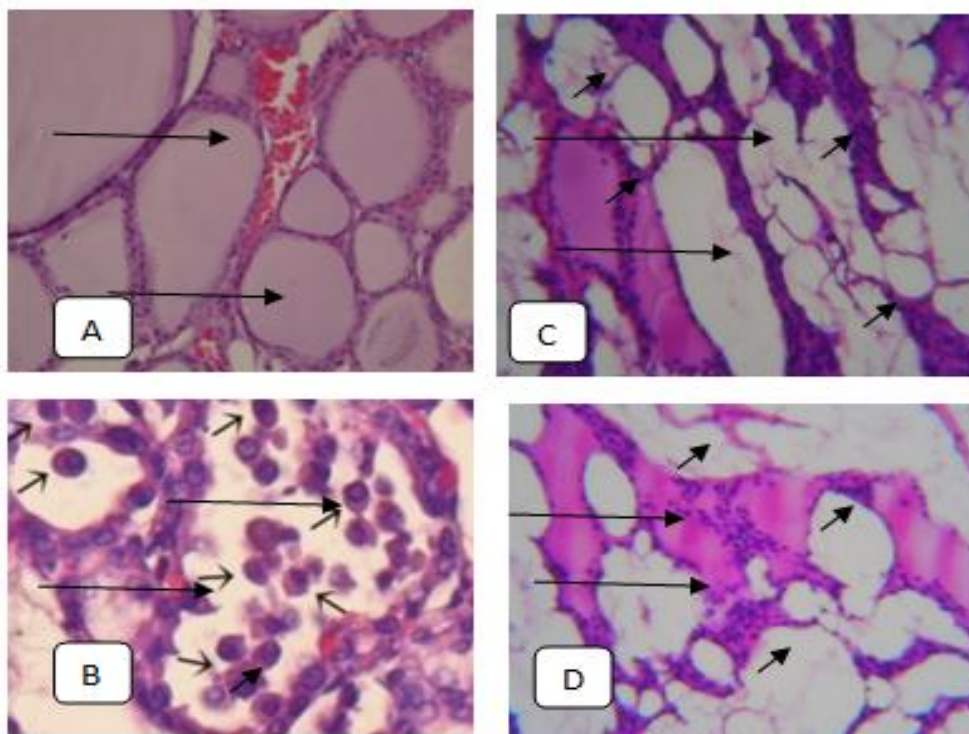


Damaging of lipid membrane by free radical substances initiated by three stages; that are initiation, propagation, and termination. Initiation process is the process when a hydrogen atom is removed from the lipid molecules. Some compounds can react with hydrogen atoms forming hydroxyl radical ( $\bullet\text{OH}$ ), alkoxy ( $\text{RO}\bullet$ ), peroxy ( $\text{ROO}\bullet$ ) and may also  $\text{HO}_2$  but not including  $\text{H}_2\text{O}_2$ . Membrane lipids generally are phospholipid consist of unsaturated fatty acids in which peroxidation is easily occur due to the issuance of methylene group ( $-\text{CH}_2-$ ) from the hydrogen atom contains only one electron. So, there are carbon atoms with no pair of electrone. The existence of a double bond in the fatty acid weakened the CH bonds on the carbon atom adjacent to the double bonds. It eased the transfer of a hydrogen atom [6]. When there is sufficient oxygen concentration lipid radicals, then it react with the oxygen to form a peroxy radical ( $\text{ROO}\bullet$ ). This formation occurs in the propagation stage. At the termination, peroxy radical ( $\text{ROO}\bullet$ ) attacks the other hydrogen atoms originating from other lipid molecules that are close by and produce lipid peroxides and peroxy radicals or interact with other antioxidants [7]. This process causes the adipose membrane compliers cells dead, and thus damaging the adipose membrane.

### Effect at $\text{KIO}_3$ and KI salt on the histological features

Treatment of  $\text{KIO}_3$  and KI salt using dose of 80 mg/Kg BW theoretically can reduce the formation of thyrosit. This is a compiler for building of the thyroid follicle wall. The affect of both salts is displayed histologically can be observed (**Figure 4**). For normal conditions, (**Figure 4-A**), healthy rat without exposure thiocyanate. The surfae of thyroid gland seem good, flat and orderly. Thyrosit which is compose of thyroid follicular wall constituent regular, normal lumen with HE staining give pink color. The parafolecular cell or cells located outside the thyroid follicles. It shows the condition of the thyroid histology in normal or healthy condition. Contrastly, histology for geitrogenic rat appear different (**Figure 4-B**), thyroid histological changing was observed. Thyrosit was not in regullar structure. It

did not surround the thyroid follicles. The feature was not clear between thyrocytes and parafollicular cells or cells itself where the position of thyrocytes and parafollicular follicles along both outside and inside of the follicle. This damage is caused by the formation of peroxides, thus increasing the production of free radicals. These free radicals trigger the formation of antibodies by modifying the protein aggregates that activate phagocytic cells to cause inflammation or injury. The formation of antibodies itself against the auto-antigens or antigens from infectious genes (including goiter factors), and that lead to formation of immune complexes and lead to complex and activation of phagocytic [8].



**Figure 4.** HE Staining results on Goitrogenic Thyroid Gland Rat (Healthy rat, A), rats exposed to goiter KSCN (Sick rat, B). Salt therapy rats with  $\text{KIO}_3$  (Therapy, C), and salt therapy with KI (Therapy, D). (Picture was recorded in 100x magnification, and arrows indicate the occurrence of structural changes in thyroid follicle, lumen, thyrocyte and parafollicular thyroid cell on treatment).

Histology appearance from the thyroid gland of the goitrogenic rat got treatment with  $\text{KIO}_3$  and KI salt was displayed in **Figure 4-C** and **D**. Both pictures gave features of thyrocytes surrounding thyroid follicles. Compared to the previous image (A and B), the parathyroid cells and thyrocytes inside the thyroid follicles were not bright, slightly darker than that in healthy thyroid gland (**Figure 4-A**). Slight damage was still observed, but improvement is indicated compared to the thyroid gland from goitrogenic rat (**Figure 4-B**). This irregularity of structures is possibly due to autoimmune processes of the body. Immune responses in the body protect it to eliminate antigens into the body and this recognizes the thyroid proteins as auto-antigens. Then, recognized of auto-antigen causes T-cell proliferation and results in the damage of thyroid tissue. Pro-inflammatory cytokines include IL-1, IL-6 and IL-8 in the thyroid gland and

appears on the body's immune response (TNF- $\alpha$ , IFN- $\gamma$ ,) causing the macrophage-mediated cell destruction occurs as well [9] [10]. This tissue damage was observed during of apoptosis on thyroid cells. It was indicated by irregularity pattern of cells, and there was thyrositis and parathyroid cells in the same feature on the thyroid follicles.

## CONCLUSION

Goitrogenic rats treated with KIO<sub>3</sub> and KI salt increase the levels of iodine (I<sup>-</sup>). Treatment with KIO<sub>3</sub> increase to 44.44% while therapy with KI reach to 56.79%. Moreover, therapy with KIO<sub>3</sub> and KI in rats exposed goitrogenic (KSCN) also decrease the malondialdehyde (MDA) level to 33.62% and 37.02%, respectively. The histological features indicated improvement on goitrogenic rat gland thyroid after therapy with both substances.

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