

Effectivity of Both KIO_3 And KI Salt Toward Iodium (I_2) Level in Urine, Malondialdehyde (MDA) And Histopathology of Thyroid Gland of Goitrogenic Rats

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Abstract

Goitrogenic a substance that can inhibit the taking of iodine by the thyroid gland, so that the concentration of iodine in the thyroid to be low., is characterized by the inflammation in the gland thyroid area caused an excessive of free radicals. An excessive of free radicals in the body cause oxidative stress, That increasing the levels of malondialdehyde (MDA) as an indicator of lipid peroxidation and decreased levels of urinary iodine excretion levels (EIU). The treated to give KIO_3 and KI salt was intended to determine the level of supplementation of iodine (I_2), the level of MDA in serum and histological rat thyroid gland. MDA levels are determined through a TBA test (Thio Barbituric acid), meanwhile the histological of the rat thyroid gland was determined by Hematoxylen-Eosin staining (HE). The results showed the KIO_3 and KI salt was significantly ($p < 0.01$) reduce levels of MDA in the serum of treatment with KIO_3 salt (33.62%) and KI salt (37,02%) and improving histological of the thyroid gland rats.

Key word: *Goitrogenic, Iodium, Malondialdehyde, KIO_3 and KI salt therapy.*

1. INTRODUCTION

Results of a national survey in Indonesia in 1998 showed 53.8 million people live in areas of iodine deficiency risk, 20 million suffer from goitre, 290 thousand estimated to suffer from creatine and creatine 9 thousand infants born each year [1]. Thyroid disease in the country continues to grow as Americans that approximately 17,000 cases occur each year and about 1,700 of them resulting in death. In the autopsy studies show that the frequency of thyroid nodules by 50% and 40% anything correlation to do with non-thyroid diseased. Study in England (*Whickham Study of the United Kingdom*) goitre is obtained 16% of the population. In the Framingham study, the ultrasound examination of thyroid nodules found in older men over 60 years at 3%, while the 48-year-old woman has a 36% of thyroid nodules. In America the majority of cases tiroiditas goitre caused by autoimmune (eg Hasimoto's disease). At the global level the most common cause of goiter due to iodine deficiency. It is estimated that about 200 million goitre among the 800 million people suffering from iodine deficiency. In the Whickham

study 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 causes disease in an inflammatory process and degradation. An inflammation of the membrane lining the gland thyroid (adiposa) caused by cytokine pro-inflammation and the high production of free radicals. The resulting of free radicals cause the onset of oxidative stresses, in which case an imbalance between of free radicals generated by antioxidant in the body so damage the cell membrane which is characterized by increased levels of malondialdehyd (MDA), one of the indicators of lipid peroxidation. So take antioxidant compounds that come from outside the body in order to suppress the occurrence of elevated levels of MDA.

Generally this is the level of intake of iodine in the end affect the status of iodine deficiency on iodine intake adequacy rate in the body. Prolonged iodine deficiency would interfere with the process of the formation of thyroid hormones. When the body is deficient in iodine, the concentration or amount of the hormone thyroxine in the blood is low. Low levels of thyroxine would stimulate the body to increase the thyroid tissue. The result is enlargement of thyroid gland is called a goiter [3]. Giving salt KIO_3 and KI to increase urine iodine status and as a source of iodine (I_2), the iodine content in urine, and lower levels of malondialdehyde (MDA) in order to overcome the interference of the thyroid gland in rats exposed goitrogenik thiocyanate (KSCN).

2. EXPERIMENT

Experimental Animals and Research Design

Experimental Animals and Research Design A number of 20 rats (*Rattus norvegicus*) (female, body weight 125-200 g) were housed at metabolic room temperature in the animal house in the Laboratory of Cellular and Molecular Biology, Mathematics and Sciences Faculty, Brawijaya University Malang and were exposed to alternate cycles of 12 h light and darkness. The rats were grouped into four group, they were a healthy group, goitrogenic group, goitrogenic group which was treated with KIO_3 salt and goitrogenic group which was treated with KI salt. The goitrogenic rats were sonde injected with 1.75 mL KSCN at the mounth with injected sonde treatment and incubated for three, six, nine, and twelve, fiveteen days to happen goiter out of iodium urine by analysis result. Then they were sonde with KSCN at mount of the rats and incubated for the next seven days. Rats were treated with the KIO_3 and KI salt at a dose of 80 mg/Kg BW of rats were given oral therapy for 14 days, and on the 15th day, rats were dissected. Rats were killed by neck dislocation, and rats serum, urine and gland thyroid were taken. The serums were taken from Then the rats be dissected in his abdomen and was taken from the serum of heart put into vacutainer non-EDTA and left for three hours and centrifuged at 600 rpm for 15 min and serum was taken for further analyzed., and the rats' gland thyroid were also taken, and the skin were slashed and washed with 0.1% NaCl and immersed in 4% PFA for seven days. All

conditions and handling of the animals were conducted following the protocols approved by Ethical Clearances Committee of Brawijaya University (238-KEP-UB).

3. PROCEDURE

Iodine (I₂) Level Measuring Using Cerric Ammonium Sulfat Test

Urine samples of 250 µg pipetted evenly shaken up in a test tube size 10 x 100 mm sealed. After that, each of the standard pipetted 0.75 mL of iodine and put into a test tube. Ammonium persulfate was added in increments of 1 mL into each test tube. After that, all the tubes are heated for 60 minutes at a temperature of 91-98 °C. The tube cooled down to room temperature before adding 3.5 mL of arsenite (As₂O₃) and homogenized with a vortex for 15 minutes. After that, add 400 mL cerric solution of ammonium sulfate (Ce (IV) NH₄SO₄) to each test tube and mixing done faster about 15 to 30 seconds time interval between the test tube by using a stopwatch. Exactly after 30 minutes, was added a solution of ammonium sulfate cerric (Ce (IV) NH₄SO₄) in the first tube, the absorbance was read using a spectrophotometer with a wavelength of 420 nm. Each tube is read at intervals of 30 seconds so the time period cerric addition of ammonium sulfate solution (Ce (IV) NH₄SO₄) to be read in each tube is 30 minutes. Iodine content can be known by an equation derived from the standard curve.

MDA levels Measurement Using Thiobarbituric Acid test

The rats serum 100 µl was added with 550 µl of aquadest, 100 µl TCA 100%, 250 µl HCl 1 N and 100 µl Na-thio 1%. The mixture was homogenized with a vortex. The mixture was centrifuged at 550 rpm for 15 minutes, and the supernatant was taken. The resulted solution was incubated in water bath at 100° C for 20 minutes, and left to room temperature and measured using UV-Vis spectrophotometer at 532 nm.

Histological Features using Hematoxylen-Eosin Staining Method

Preparate of the rats thyroid gland put into 1-3 xylol respectively for 5 minutes, and was put into the variation of ethanol which was started from absolute ethanol 1-3, ethanol 95%, 80%, and 70% respectively for 5 minutes, and was soaked in aquadest for 5 minutes. Then, it was put into hemotoxylen dyes for ± 10 minutes to penetrate the equipment color. After that, it was washed over flowing water for 30 minutes, and rinsed with aquadest before continued to a colouring with eosin dye. The colour was resulted using eosin stained by inserting the equipment into the eosin alcohol for 5 minutes, then 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 done by putting it in the xylol 1, 2 and was further dried. Finally, the result was mounted using entellan. The dried and stained ultrathin sections were observed using a microscope (Olympus BX53) with a magnification of 100 times.

4. RESULTS AND DISCUSSION

Effect Thiocyanate (KSCN) and Level of Iodine and in Rats Urine

The result of observation the impact of changes in the physiology of bodies of rat to suffer goitre due to exposure to thiocyanate (KSCN). KSCN who act as agents an inhibitor the active transport of iodide ions in the body of rat, so it will be deficient in iodine content as shown in **Figure 5.1 and Table 5.1**. Diagnosis of observation through the screening program with an evaluation of the physiological changes experienced by the rate, so it will be a little picture of changing conditions know the results of the control rats, rats experienced goitrogenik, and rats treated with a salt therapy KIO_3 and KI will be visible difference visible in white rat physiology types (*Rattus norvegicus*).

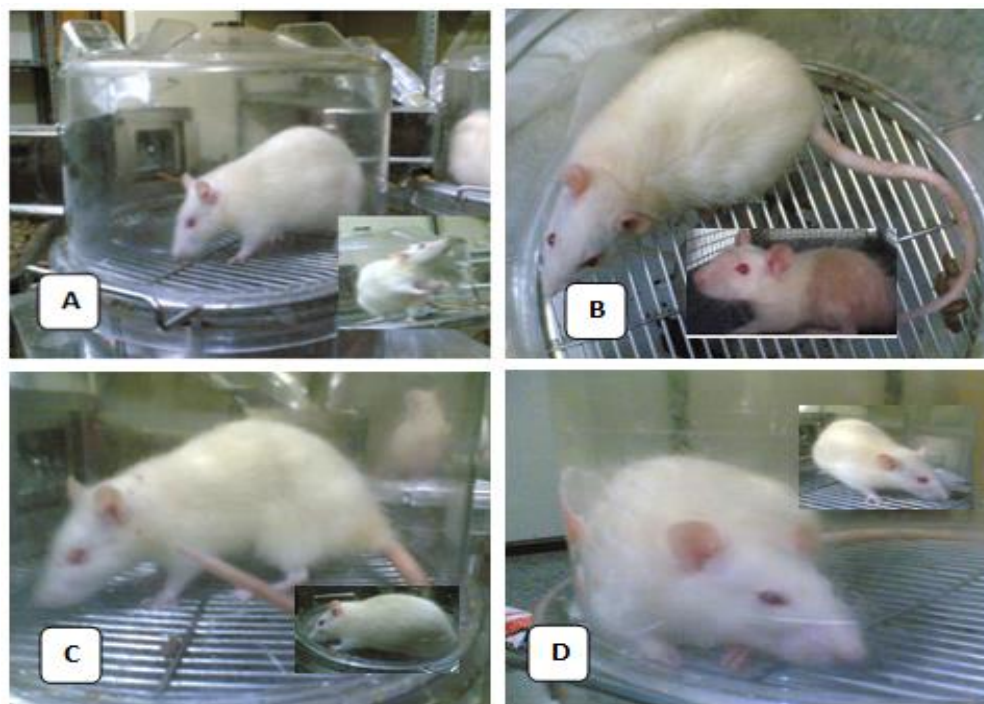


Figure 1. Normal Physiological Rat (Healtht. A), Rats Exposed to Thiocyanate KSCN (Goitrogenik Sick. B). Salt Therapy rats KIO_3 (Therapy. C), and Salt Therapy KI Rats (Therapy. D) salt and therapies rat with KI salt

There deficiency levels of iodine (I-) rat were exposed by goitrogenic thiocyanate (KSCN). The results shown in **Table.2** with goitrogenik groups of mice (positive control) (0.035 ± 0.022 ppm) is lower than the healthy group (negative control) (0.180 ± 0.011 ppm). As for the group treated with salts of potassium iodate (KIO_3) and salts of potassium iodide (KI), respectively (0.063 ± 0.008 ppm) and (0.081 ± 0.007 ppm) there was lowering pain compared with rat (positive control). This indicates the occurrence of pro-inflammatory cytokines that

increase the levels of iodine deficiency (I) in rat urine goitrogenik. Results of statistical analysis showed the influence of elevated levels of iodine in the urine of rat goitrogenic significant level ($p < 0.01$) were significantly different.

Table.2 Urine Iodine Levels

Group	Levels of Iodine Average (ppm)	Iodine Lowering levels towards Control (%)	Iodine Increasing levels towards Control (%)
Healthy (negatif control)	0,180±0,022	-	-
Sick (positif control)	0,035±0,011	80,55	-
Treatment KIO ₃	0,063±0,008	65,00	44,44
Treatment KI	0,081±0,007	55,00	56,79

It also stated that the incidence of iodine deficiency can be caused by thiocyanate KSCN inhibit iodine uptake by the thyroid gland to bind organic iodine (monoiodotirosine and diiodotirosine which is part of the triglobulin) located in each cell, causing severe competition between serum thiocyanate and thyroxine bound as the result of an increase in serum thyroxine. Salt supplementation KIO₃ and KI salts in mice with disease treatment goitrogenik helped reduce the level of iodine transport barriers that remain unfulfilled iodine content after administration of excess iodine. Bound iodine in salt KIO₃ and KI salts are converted in the form of iodide (I⁻) hormogenesis in the process of thyroid hormone, so it will increase iodine thought to be caused by inhibition of H₂O₂ generation by low due to the content of the I-intratiroidal high due to supplementation KIO₃ and KI . Giving KIO₃ and KI pituitary function reduces pacing work harder to secrete TSH will stimulate the thyroid gland to produce thyroid hormone, resulting in thyroid hyperplasia and hypertrophy are not experiencing (goiter) [4]

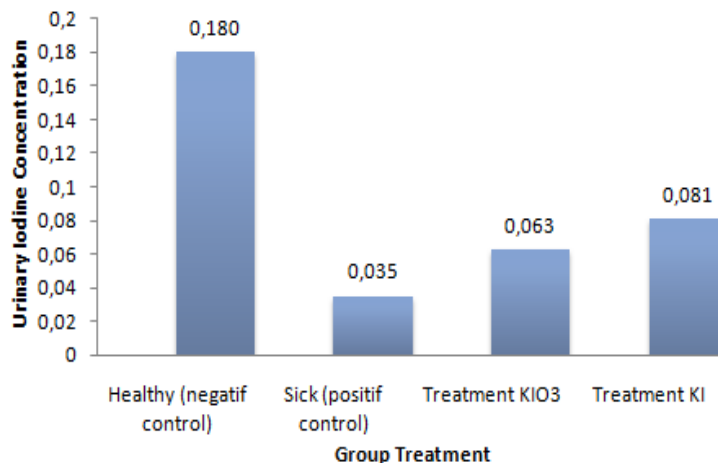


Figure 2. Comparison of the value of Urine levels of Iodine in healthy rats (*Rattus norvegicus*), goitrogenic rats, and therapies rat with KIO₃ salt and therapies rat with KI salt

Levels of Malondialdehyd (MDA) in Rats Serum

KIO₃ and KI salt can reduce levels of MDA (Table 3 and Figure 3). The results showed the increasing levels of MDA in the group of rats with goitrogenic (7.857 ± 0.041 ppm) higher than the healthy group (2.820 ± 0.014 ppm). While in the group of rats with goitrogenic treated using KIO₃ salt (5.215 ± 0.146 ppm) and group of rats with goitrogenic treated using KI salt (4.925 ± 0.095 ppm) showed the decreasing levels of MDA compared to the rats with goitrogenic It self. This indicate the therapy of KIO₃ and KI at a dose of 80 mg/Kg BW provide a positive influence on the decreasing of the levels of MDA goitrogenic rat serum. Statistical analysis results also showed a significant influence on the group goitrogenic rat serum toward the goitrogenic rat serum treated with KIO₃ and KI salt (P < 0.01).

Table 3. Malondialdehyde levels (MDA) in Rat Serum

Group	Levels of MDA Average (µg/mL)	Iodine Increasing levels towards Control (%)	Iodine Lowering levels towards Control (%)
Healthy (negatif control)	2,820±0,014	-	64,10
Sick (positif control)	7,857±0,041	178,61	-
Treatment KIO ₃	5,215±0,146	84,92	33,62
Treatment KI	4,925±0,095	74,64	37,02

MDA levels in rat serum (*Rattus norvegicus*) decreased after treatment with KIO₃ salt (33.62%) and KI salt (37,02%). Decreasing of MDA level possible was caused by an iodium substance found (antioxidant nutrient) in the KIO₃ and KI salt. Giving iodized salt potassium iodate (KIO₃) and potassium iodide salt (KI) in (*Rattus norvegicus*) goitrogenik aims to activate

iodine in the process due to the inhibition of active substances transport goitrogen thiocyanate (KSCN) so the addition of a number of iodine in the thyroid gland may increase the activity of iodine can fix secretion of thyroid hormone is triiodothyronine (T_3) and thyroxine (T_4) due to high levels of TSH in the thyroid gland and in the end there will be a reduction in tissue destruction of the thyroid gland. Theoretically, inhibition mechanism of lipid peroxidation by KIO_3 and KI salt as antioxidant compound can counteract free radicals in the system. KIO_3 and KI salt has an iodine substance (antioxidant nutrient) antioxidant content of iodine activity [5]. Compounds from iodine activity have antioxidant activity that useful to counteract free radicals. Malondialdehyde which is the product of peroxidation can be used as an indicator for occurring of a lipid disorder. Therefore, it can be concluded the high levels of malondialdehyde in the goitrogenic rat's serum as an indication of the high levels of adiposa membrane tissue disorder which were happened due to the reactive oxygen compounds.

Lipid damage consists of three phases; 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 ($\bullet OH$), alkoxy (RO), peroxy (ROO) and may also HO_2 but not including H_2O_2 . Membrane lipids generally are phospholipid consist of unsaturated fatty acids in which peroxidation is easily occur due to the issuance of methylene group (-CH₂-) from the hydrogen atom contains only one electron. So, there are carbon atoms with no pair of electron. 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 (ROO \bullet). This formation occurs in the propagation stage. At the termination, peroxy radical (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 adiposa membrane compliers cells dead, and thus damaging the adiposa membrane.

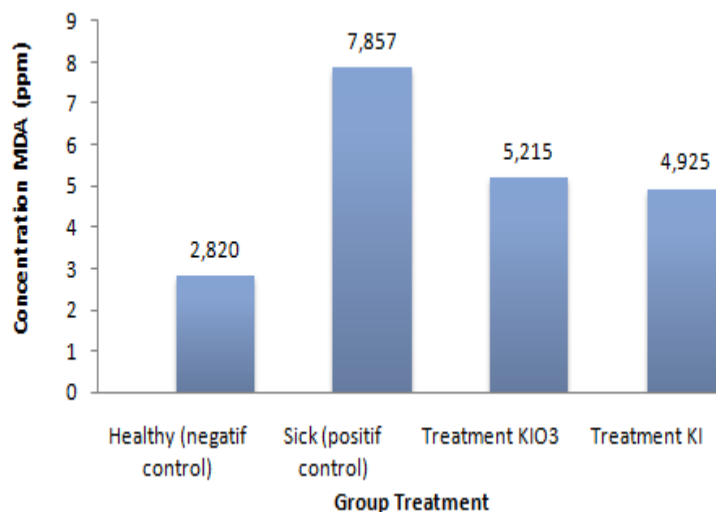


Figure 3. Comparison of the value of Serum levels of MDA in healthy rats (*Rattus novergicuss*), goitrogenic rats, and therapies rat with KIO₃ salt and therapies rat with KI salt

Effect at KIO₃ and KI Salt toward Histological Features of Goitrogenic

The results in Figure 2 showed that the KIO₃ and KI Salt at a dose of 80 mg/Kg BW reduce the formation of tirosit which is a compiler of the thyroid follicle wall composed Figure 5.5 (A) shows normal conditions without exposure to the thyroid gland thiocyanate where the surface looks flat and orderly. Comparisons between groups of images 5.5 (B) and (A) shows the histological changes in the thyroid. Group picture 5.5 (A) shows tirosit which is composed of thyroid follicular wall constituent regular, normal lumen with HE staining uses colored pink and parafolikular cells or cells located outside the thyroid follicles. It shows the condition of the thyroid histology in normal conditions. Group picture 5.5 (B) shows goitrogenik group. Based on the drawing 5.5 (B) it can be seen that there tirosit been irregular. Tirosit not surround the thyroid follicles again and it is not clear between tirosit with parafolikular cells or cells where the location tirosit parafolikular follicles located along both outside and inside the follicle. Damage is caused by the formation section Panus thus increasing the the production of free radicals. These free radicals can trigger the formation of antibodies, by modifying the protein aggregates that can activate phagocytic cells and cause inflammation. The formation of antibodies against autoantigens or antigens from infectious genes (including goiter factors) can lead to the formation of immune complexes which in turn can lead to complex and activation of phagocytic [8]. While drawing a picture of the thyroid group 5.5 (C) and (D) the group of goitre and therapy group showed tirosit KIO₃ and KI were surrounding thyroid follicles approaching histological picture group 5.5 (A). In addition, histopathological picture image group 5.5 (C) and (D) shows the parathyroid cells and tirosit inside the thyroid follicles but had no lumen darker than the picture group 5.5 (A). Thyroid tissue damage that is caused by the autoimmune processes of the body. Immune response in the body's defense mechanisms to function in a manner that destroys

antigens into the body recognizes the thyroid proteins as autoantigens. Furthermore, autoantigen recognized cause T cell proliferation and result in damage to the organ of thyroid tissue. Proinflammatory cytokines include IL-1, IL-6 and IL-8 in the thyroid gland and appears on the body's immune response (TNF- α , IFN- γ ,) causing the macrophage-mediated cell destruction occurs [9] [10]. This tissue damage can be seen with the occurrence of apoptosis of thyroid cells are characterized by irregular layout cells and there tiroisit and parathyroid cells in the lumen of the thyroid follicles.

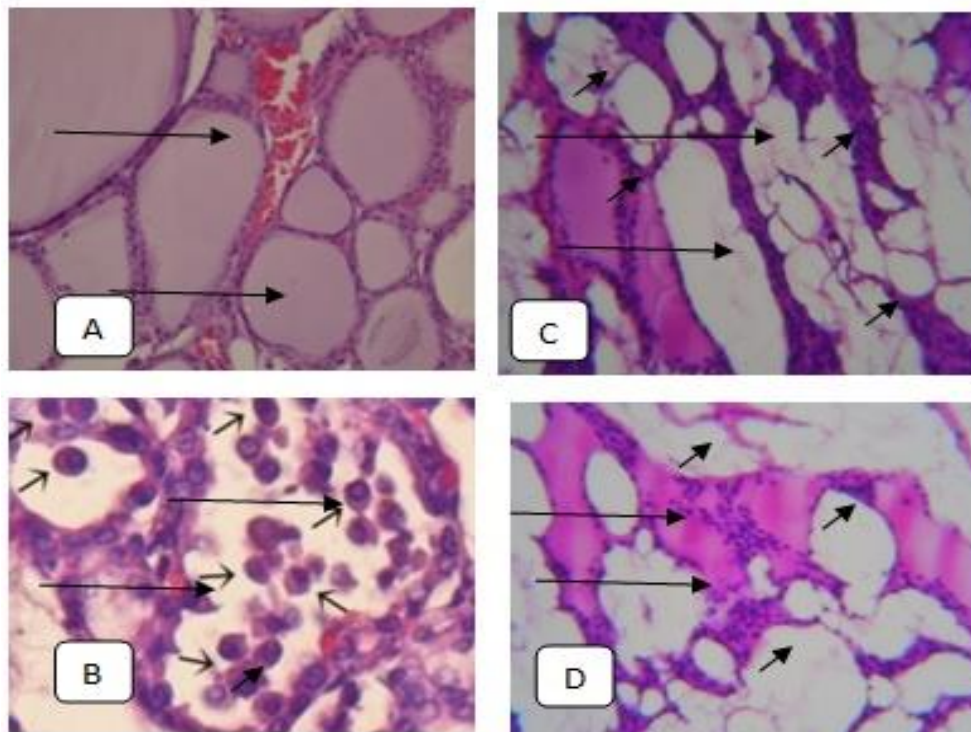


Figure 4. HE Staining results on Goitrogenic Thyroid Gland Rat (Healtht. A), Rats Exposed to Goiter Thiocyanate KSCN (group Sick. B). Salt Therapy rats KIO_3 (Therapy. C), and Salt Therapy KI Rats (Therapy. D) salt and therapies rat with KI salt. A 100x magnification. Arrows indicate the occurrence of structural changes in tyroid folikel, lumen, tiroisit and parafolikular tiroid cell on treatment

5. CONCLUSION

Based on the research conducted on goitrogenic rats treated with KIO_3 and KI salt, it was found that KIO_3 and KI salt therapy with dose of 80 mg/Kg BW Therapy KIO_3 and KI in rats exposed goitrogenic (KSCN) showed increasing levels of iodine (I-) of 44,44% after treated KIO_3 while therapy KI was 56,79% and Therapy KIO_3 and KI in rats exposed goitrogenic (KSCN) showed decrease levels of malondialdehyde (MDA) of 33.62% for the therapy, while treatment with KIO_3 and KI at 37, 02%. Showed histological of goitrogenic rat gland thyroid.

ACKNOWLEDGMENT

The author would like to thank to the head and staff Laboratory of Animal Physiology Islamic University of Malang, thank to the head and staff of Biochemistry Laboratory of Faculty of Sciences The University of Brawijaya along with laboratory staff who have helped in this research.

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