

The Protective Effect of Vitamin E and Selenium on Methimazole-induced Hepato-renal Toxicity in Adult Rats

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Abstract: Background: Methimazole (MMI) is a drug used to manage hyperthyroidism associated with grave's disease. Liver and kidney toxicity are the main side effects when using MMI which may be ameliorated by antioxidants. **Aim:** Investigate the protective effect of antioxidant Vitamin E (Vit. E) and/or Selenium (Se) supplementation on MMI- induced hepatotoxicity and nephrotoxicity in adult rats. **Methods:** Thirty male rats divided into 5 groups of 6 each: group I received corn oil + water; group II received corn oil +MMI; group III received Vit.E + MMI; group IV received Se+corn oil+MMI; group V received Vit.E(100 mg/kg) + Se(0.1 mg/kg)+ MMI (60mg/kg BW) for 28 days via gavage. At the end of the experimental period, studied the histology of the liver and kidney tissues, biochemical measurement of different parameters of liver and kidney function tests and the determination of the antioxidant enzyme activities. **Results:** Rats exposed to MMI showed significant decreased in plasma uric acid (URCA) level, and liver and kidney Superoxide dismutase (SOD) than negative control group. While plasma creatinine (CREA), transaminases (AST and ALT), lactate dehydrogenase (LDH) activities, total bilirubin (TBILI) and alkaline phosphatase (ALP) levels were higher than negative control group. Co-administration of Vit. E and Se improved that parameters cited above. The biochemical results were confirmed by the histopathological findings in liver and kidney. **Conclusion:** The combination of Vit.E and Se is more effective in ameliorating MMI-induced hepatotoxicity and nephrotoxicity than either antioxidant alone in rats.

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1. Introduction:

The present study highlights the indirect yet important role of nutrition in the treatment protocol of disease. In medicine very often pharmaceutical treatments of a particular disease may cause negative side effects which may or may not be related to the original disease. Adding specific nutrients to the treatment protocol may reduce these negative side effects. In this case we investigated the role of antioxidant nutrients, Vitamin E (Vit. E) and Selenium (Se), in reducing liver (hepatotoxicity) and kidney toxicity (nephrotoxicity) which are major negative side effects when using Methimazole (MMI) to treat Graves disease. Graves' disease usually begins after age of 20 at a female-to-male ratio of 5:1; and it is characterized by hyperthyroidism (Kahaly *et al.*, 2011). MMI is an anti-thyroid drug commonly prescribed for individuals affected by hyperthyroidism associated with Grave's disease (Cooper, 1999, Koornstra *et al.*, 1999). MMI is absorbed by the gastrointestinal tract and concentrated in the thyroid gland to inhibit the production of thyroid hormone (Aboul-Enein and Al-Badr, 1979). The aim of this study was to investigate the protective effect of antioxidant Vit E and/or Selenium Se supplementation on MMI- induced hepatotoxicity and nephrotoxicity in adult rats.

2. Methodology:

Animals.

Thirty Adult Wistar rats, weighing about 190-220g, were purchased from the animal house of King Fahd Research Center at King Abdul Aziz University, Jeddah, KSA. They were housed at ambient temperature 22±3 °C in a 12-h light/dark cycle and a minimum relative humidity of 40%. The experiment was conducted in accordance with King Fahd Research Center institutional guidelines for the care and use of laboratory animals.

Commercial standard diet (Grain soils and flour mills organization, SA) (Table1) and tap water were given *ad libitum*. The concentration of selenium in standard diet (0.1 mg/kg of diet) was determined, after mineralization, by the Electrothermic Atomic Absorption Spectrometer with a 196 nm wavelength (Endreffy *et al.*, 1991). All rat groups also fed corn oil purchased from commercial suppliers in Saudi Arabia.

Chemicals.

Methimazole (C₄ H₆N₂S), sodium selenite (Na₂ SeO₃), and vitamin E (α -tocopherol acetate) were purchased from Sigma Company (USA). All other chemicals were purchased from standard commercial suppliers in Saudi Arabia.

Table 1: Nutrient composition of commercial standard diet/ 100 g.

Nutrient	Amount	Nutrient	Amount
Crude Protein, %	20	Calcium, %	1
Crude Fat, %	4	Phosphorus, %	0.60
Crude Fiber %	3.50	Vitamin A, IU/g	20
Ash, %	6	Vitamin D, IU/g	2.20
Salt, %	0.50	Vitamin E, IU/kg	70
Energy, Kcal/Kg	2850		

Experimental design.

After one-week of acclimatization into the laboratory conditions, 30 rats were randomly divided into five groups of six rats each.

Group 1: Corn oil, 30min later water (negative control)

Group 2: Corn oil, 30min later MMI (positive control)

Group 3: Vit.E, 30min later MMI

Group 4: Se + corn oil, 30min later MMI

Group5: Se + Vit. E, 30min later MMI

Selenium and Methimazole were dissolved in water (0.2ml/day), while vitamin E was dissolved in corn oil (0.2ml/day), all those treatments given by gavage. The dose of MMI (60mg/kg body weight/day) was chosen according to (Cano-Europa *et al.*, 2011) where MMI induced the classical picture of hypothyroidism without lethal effects. The selenium dose (0.1 mg/kg body weight/day) and Vitamin E dose (100mg/kg body weight/day) were used in this study which exhibited high protection against toxicity as described in previous studies (Stajn *et al.*, 1997, Patra *et al.*, 2001, El-Demerdash *et al.*, 2004 and Aboul-Soud *et al.*, 2011).

Feeding protocol was carried out for four weeks (28 days). At the end of the experimental period (4 weeks), animals in all groups were sacrificed under ether anesthesia then the liver and kidney weight taken and calculated for relative weight and body weight changes as a mean for each group.

Blood was collected from all rats into heparianized tubes over night fasting from the aortic puncture of rats, centrifuged at 2200 r.p.m for 10 min. Plasma samples were obtained and stored at -80oc until biochemical analysis.. Livers and kidneys from male rats of different groups were dissected out, cleaned and weighted. Some samples were rinsed, homogenized (10%, w/v) in an appropriate buffer (pH=7.4) and centrifuged. The supernatants were used for biochemical assays. Other samples were prepared for histological examination.

Biochemical estimations.**Determination liver and kidney functions plasma.**

Plasma levels of liver function tests: aspartate aminotransferase (AST) and alanine aminotransferase

(ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and bilirubin (BIL) and plasma levels of kidney function tests: creatinine (CREA) and uric acid (URCA) were tested at the hospital of clinical laboratories of the king Abdul Aziz University.

Determination of antioxidant enzyme activitie.

The antioxidant enzyme activity was determined in kidney homogenates diluted in phosphate buffer by measuring the superoxide dismutase activity according to the method described by (Beutler, 1984). One hundred microliters of the prepared supernatant was mixed with 1.5 ml of a Tris-HCl buffer (pH 8.5) and 1000 µl of 15 mM pyrogallol and then incubated at 25°C for 10 min. The reaction was examined by adding 50 µl of 1 N HCl and the absorbance will be measured at 440 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activities were expressed as U/ml protein. Was measured by kit ELISA (Cayman, USA, Ref; 706002)

Histological studies.

Liver and kidney samples of about 0.5 cm³ were taken and immediately fixed in formalin (10%) solution for 48 h and processed in a series of graded ethanol, then embedded in paraffin, serially sectioned at 3 µm and stained with hematoxylin-eosin stain for light microscopy examination.

Statistical analysis.

In each assay, the experimental data represented results were expressed as means of six independent assays (n=6) ± standards deviations (S.D.). The data was analyzed using the statistical package program for Social Sciences (SPSS) Version 16. Statistical analysis was performed using one-way Analysis of Variance (ANOVA). Multiple comparisons between means were performed using Bonferroni test. Differences were considered significant at the level $p < 0.05$.

3. Results:**Enzymatic antioxidant status in liver and kidney.**

In the liver and kidney homogenates of MMI-treated rats, superoxide dismutase (SOD) activities decreased significantly 61% and 81% respectively in male rats, when compared to controls (Table 2). Supplementation of Vitamin E regenerated SOD activity in (MMI + Vit. E) group when compared to MMI-group. However, Selenium supplementation in the diet of (MMI + Se) group partially ameliorated SOD activity. The administration of Se or Vit. E in MMI group resulted to high SOD activities less than Vit. E +Se + MMI group. Additionally, administration of selenium and vitamin E in the MMI group resulted to high SOD activities more than MMI group ($p < 0.05$).

Table 2: Kidney and Liver hemogenates of Superoxide dismutase (SOD) levels in control group, treated groups with Methimazole (MMI), Vitamin E (Vit.E)+ Methimazole (MMI), Selenium (Se) + Methimazole (MMI) or Vitamin E (Vit. E)+ Selenium (Se) +Methimazole (MMI)

Parameters and treatments	LSOD*	KSOD*
	U/ml protein	U/ml protein
Control	1344.08 ±93.63	138.77 ±9.10
MMI	816.41 ±74.17 ^{a,c,d}	112.22 ±9.89 ^{a,c}
Vit E + MMI	1153.51 ±84.12 ^b	122.41 ±10.23 ^b
Se+ MMI	1049 ±65.32 ^b	114.16 ±9.42 ^a
Vit E + Se + MMI	1252.75 ±64.86	134.32 ±11.88

* **LSOD:** Liver superoxide dismutase, **KSOD:** kidney superoxide dismutase.

(^a $p < 0.001$ compared to control group, ^b $p < 0.05$ compared to control group, ^c $p < 0.01$ compared to Vit E + MMI & Vit E + Se + MMI groups, ^d $p < 0.05$ compared to Se+ MMI)

Liver biochemical markers (AST, ALT, ALP, LDH and TBIL levels) in plasma:

The effect of MMI on biochemical markers in plasma (ALT, AST activities, ALP, LDH and total bilirubin levels) showed 202%, 143%, 191%, 174%

and 225% respectively, when compared with those of controls (Table 3). Supplementation of selenium and/or vitamin E in the diet of the MMI-treated group restored all the parameters cited above.

Table 3: Plasma levels of liver function in control group, treated groups with Methimazole (MMI), Vitamin E (Vit.E)+ Methimazole (MMI), Selenium (Se) + Methimazole (MMI) or Vitamin E (Vit. E)+ Selenium (Se) +Methimazole (MMI)

Parameters & treatments	AST	ALT	ALP	LDH	TBIL
	U/L	U/L	U/L	U/L	mg/dl
Control	26.83 ±3.60	37.33 ± 4.13	62.00 ±8.32	94.17 ±7.68	2.00 ±0.63
MMI	54.17 ±5.67 ^a	53.33 ± 4.32 ^a	118.33 ±11.31 ^a	163.83 ±16.77 ^a	4.50 ±0.84 ^a
Vit E + MMI	33.17 ±2.86 ^c	39.17 ± 4.88 ^d	68.67 ±6.71 ^c	107.67 ±12.60 ^d	2.67 ±0.82 ^d
Se + MMI	37.50 ± 3.39 ^{b,d}	41.50 ± 4.23 ^d	73.17 ±9.90 ^d	110.17 ±16.47 ^d	3.33 ±0.82
Vit E + Se +MMI	28.83 ± 4.36 ^c	38.50 ± 4.59 ^c	63.50 ±7.01 ^c	99.17 ±10.93 ^c	2.33 ±0.82 ^c

AST: aspartate aminotransferase, **ALT:** alanine aminotransferase, **ALP:** alkaline phosphatase, **LDH:** lactate dehydrogenase, **TBIL:** Total bilirubin

(^a $p < 0.001$ compared to control group, ^b $p < 0.05$ compared to control group, ^c $p < 0.01$ compared to methimazole ^d $p < 0.05$ compared to methimazole)

Kidney function markers (URCA and CREA levels) in plasma:

URCA level in the plasma of the MMI- treated group decreased by 46% when compared with those of negative controls (Table 4). CREA level increased by

174% in the plasma of the MMI- treated group when compared with those of controls. Supplementation of selenium and/or vitamin E in the diet of the MMI-treated group restored all the parameters cited above.

Table 4: Plasma levels of kidney function in control group, treated groups with Methimazole (MMI), Vitamin E (Vit.E)+ Methimazole (MMI), Selenium (Se) + Methimazole (MMI) or Vitamin E (Vit. E)+ Selenium (Se) +Methimazole (MMI)

Parameters and treatments	URCA	CREA
	mg/dl	mg/dl
Control	50.00 ±4.73	32.50 ±3.08
MMI	22.83 ±2.23 ^a	56.50 ±5.36 ^a
Vit E +MMI	44.33 ±4.03 ^d	39.17 ±4.96 ^c
Se +MMI	32.17 ±3.49 ^{b,d}	44.50 ±6.09 ^{b,d}
Vit E + Se + MMI	48.17 ±5.53 ^c	35.83 ±4.75 ^c

URCA: uric acid, **CREA:** Creatinine.

(^a $p < 0.001$ compared to control group, ^b $p < 0.05$ compared to control group, ^c $p < 0.01$ compared to methimazole ^d $p < 0.05$ compared to methimazole)

Histological studies:

Light microscopic examination indicated a normal structure of the liver in the controls (Fig.A.1). The

exposure of rats to MMI induced degenerative changes in portal spaces, hepatocytes and central area of liver lobules. MMI caused changes in portal spaces: dilation, abnormal branching, filled congestion red blood cells and inflammatory infiltrative cells. Changes in hepatocytes: vacuolated, eccentric nuclei and indistinct boundaries. Also changes in central area of liver lobule: loss of cellular continuity, loss of radial distribution hepatocytes, dilation of the blood sinusoids, focal areas of parenchymal necrosis and inflammatory cellular infiltrates (Figs B 3,4 &5). Co-administration of Se or vitamin E revealed regression of the histological changes. In the MMI + Vit. E group, we noted some portal spaces appeared dilated with connective tissue deposition, vacuolated cytoplasm and dilatation and congestion of the central veins with dilatation of the blood sinusoids and some aggregates of kupffer cells (Figs. C 6 &7). But in the MMI+ Se group, noted dilation in some portal spaces, portal veins, peri-central blood sinusoids and central veins (Figs. D 8 &9). Sever liver damages, observed in MMI group, significantly ameliorated in (MMI + VitE + Se)-treated group (Fig. E 10 & 11). The histological pattern was nearly normal in rats treated only with selenium and vitamin E, Dilation of some portal spaces with congested portal veins were seen in some hepatic lobules. Dilated and congested peri-central blood sinusoids were also observed. Moreover, most of the hepatocytes appeared normal.

On the other hand, light microscopic examination indicated a normal structure of the kidney in the controls (Fig.2). The exposure of rats to MMI induced degenerative changes in glomeruli and in convoluted tubules. MMI caused changes in some renal corpuscles showed hypertrophy of the glomerular capillaries tuft with decrease of the capsular space while in others; the glomeruli appeared retracted leaving a wide capsular space. Some glomeruli showed dilated peripheral capillary loops with vacuolization, and some showed hyaline deposition in the form of eosinophilic material in between their capillary loops. Regarding the proximal convoluted tubules, some tubules showed thickening of the basement membrane with their lining cells appeared swollen, vacuolated and bulged into the lumen indicating.... While other tubules showed massive atrophy with disappearance of the brush borders. Meanwhile, widening of the lumen of the distal convoluted tubules with flattening of their epithelial lining was obvious with some pyknotic nuclei (Figs. B 14,15). Co-administration of Se or vitamin E revealed regression of the histological changes. In the MMI+ Vit. E group, we noted some glomeruli showed a localized deposition of eosinophilic hyaline material with dilatation of the peripheral capillary loops. The proximal and distal convoluted tubules appeared more or less normal while

others showed dilatation with loss of brush border (Fig. C 16). But in the MMI+ Se group, noted congestion and dilatation of capillary loops. The proximal and distal convoluted tubules appeared more or less normal while others showed less evidence of damage in the form of small vacuolation or loss of brush borders (Fig. D 17). Sever liver damages, observed in MMI group, significantly decreased in (MMI+VitE+Se)-treated group(Fig. E 18). The histological pattern was nearly normal in rats treated only with selenium and vitamin E, Some of the proximal and distal convoluted tubules appeared more or less normal while others showed less evidence of damage in the form of small vacuolation or loss of brush borders.

4. Discussion:

Liver and kidney play a major role in detoxification process, hence our choice to test for liver and kidney parameter and histological examination. Our results affirmed the notion that MMI treatment induced liver toxicity shown by increased activity ($P<0.001$) in ALT, AST, ALP, LDH and TBILI in plasma (202%, 143%, 191%, 174% and 225%) of male rats in the positive control group. These elevated levels indicated overall balance disturbance between the degree of oxidative stress and the antioxidant capacity (Cano-Europa, *et al.*, 2011). These results are agree with other studies (Ramos-Bonner *et al.*, 2007; Chen *et al.*, 2009; Gallelli *et al.*, 2009) which indicated that MMI could cause liver damage.

Supplementation with Vitamin E + Selenium as a combination significantly (53.2%, 72.2%, 53.7%,60.5% and 51.8%) improved liver function parameters by reducing activities of AST, ALT, ALP, LDH and TBILI enzymes ($P<0.01$). Whereas supplementation with only Vitamin E showed lower significance (61.2%, 73.4%, 58%,65.7% and59.3% respectively)($P<0.05$) in parameters. Also supplementation with only Selenium showed lower significance (69.2%, 77.8%, 61.8% and 67.2% respectively) ($P<0.05$) in four of the parameters (AST, ALT, ALP and LDH).

More evidence indicating kidney toxicity was shown by an increase in the activities of CREA and decrease in URCA in plasma of male rats in the MMI treated group (45.7% and 173.8% respectively) ($p<0.001$). Supplementation with Selenium significantly (78.8% and 140.9% respectively) ($P<0.05$) improved kidney function parameters by increasing CREA activity and reducing URCA activity levels compared with those of MMI treated rats. These results agree with Ben Amara *et al.*, who mentioned that MMI could cause kidney damage. Supplementation with vit E + Se in combination showed a (63.4% and 210.9% respectively) ($P<0.05$) significance.

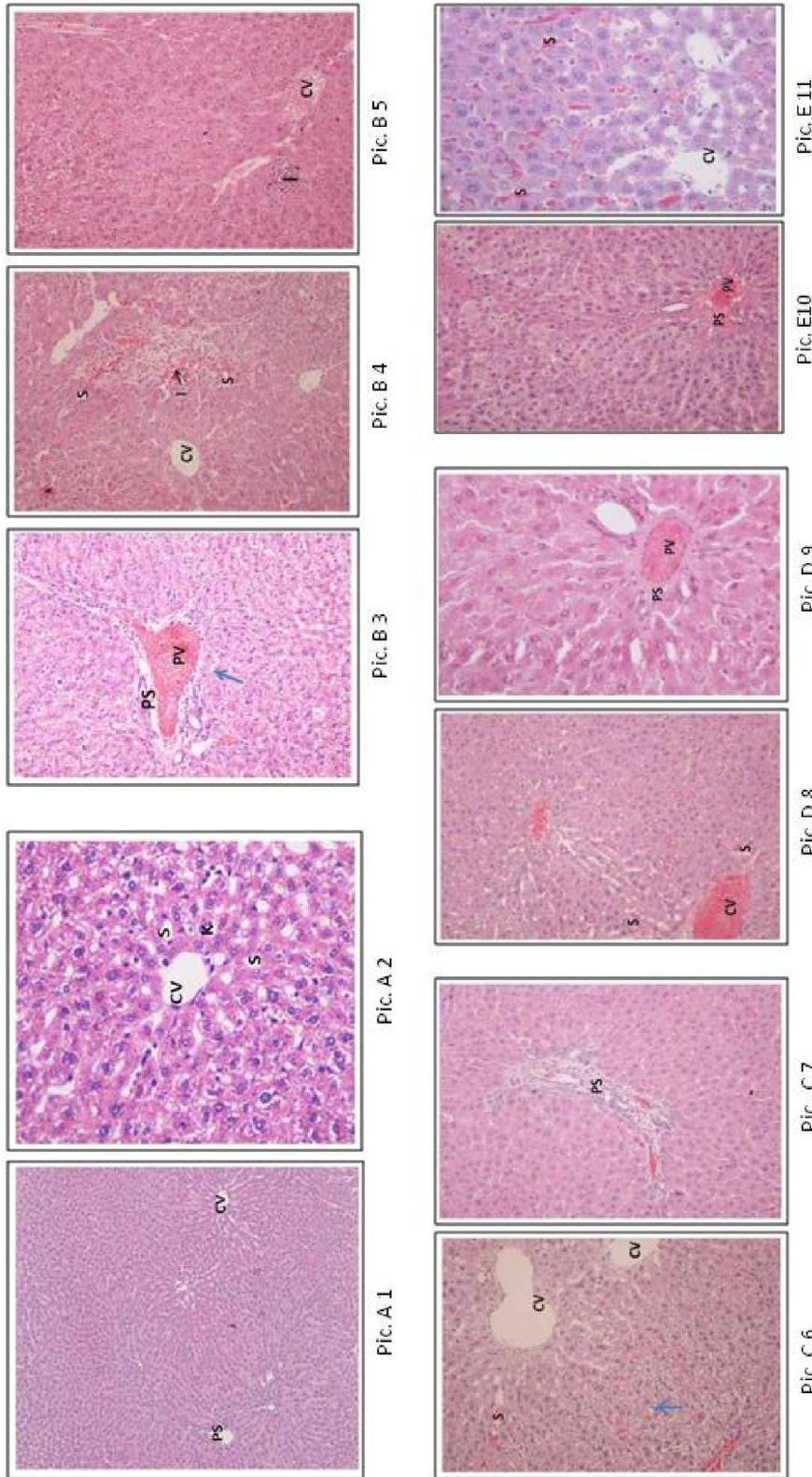


Fig 1: Histological findings in liver tissue of adult rats from the 5 experimental groups: (A 1,2) Control group—normal lobular hepatic architecture; (B 3, 4 , 5) methimazole group—showed changed in portal spaces, hepatocytes and central area of liver lobule (indicated by arrows); (C 6,7) methimazole + Vitamin E group—histological damage decreased (D 8, 9) methimazole + selenium group histological damage decreased; (E 10, 11) methimazole + vitamin E+ selenium group—showed nearly to the normal liver picture as in control group. Arrows indicate: (CV) central vein; (PS) portal spaces; (S) sinusoids; (K) kupffer cells; (PV) portal vein; (↓) infiltrations (↓) vacuolated hepatocytes.

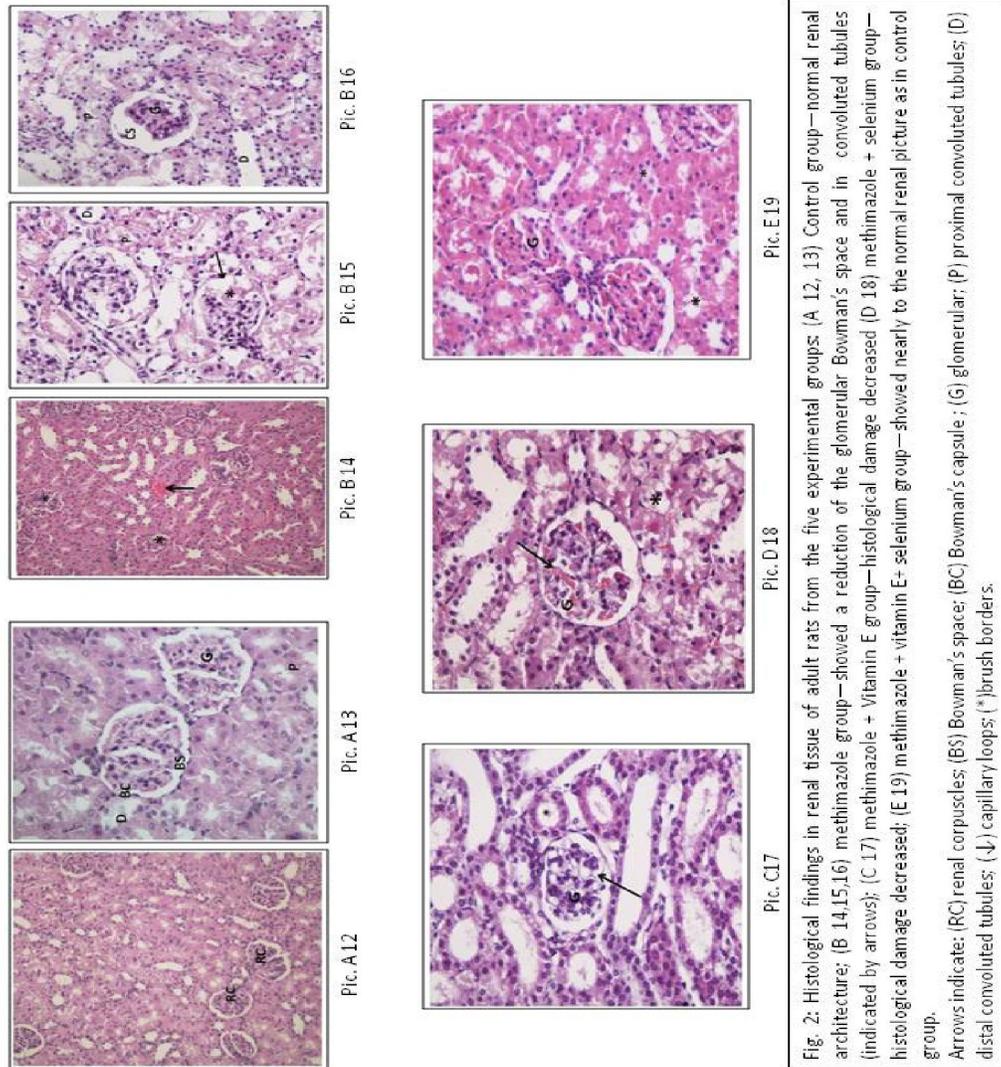


Fig. 2: Histological findings in renal tissue of adult rats from the five experimental groups: (A 12, 13) Control group—normal renal architecture; (B 14,15,16) methimazole group—showed a reduction of the glomerular Bowman's space and in convoluted tubules (indicated by arrows); (C 17) methimazole + Vitamin E group—histological damage decreased (D 18) methimazole + selenium group—histological damage decreased; (E 19) methimazole + vitamin E+ selenium group—showed nearly to the normal renal picture as in control group.
Arrows indicate: (RC) renal corpuscles; (BS) Bowman's space; (BC) Bowman's capsule; (G) glomerular; (P) proximal convoluted tubules; (D) distal convoluted tubules; (↓) capillary loops; (*) brush borders.

Superoxide dismutase (SOD) is antioxidant enzyme which deactivates superoxide free radicals and defense control oxygen. It catalyzes the dismutation of superoxide free radicals (Fridovich, 1972). In our study, we found that the activity of SOD in liver and kidney were significantly lower in MMI(60.7% and 80.9% respectively) ($P<0.001$). Supplemented MMI group with Vitamin E and Selenium improve SOD in both liver and kidney. Combination Vit. E and Se significantly lower (153.4% and 119.7% respectively) ($P<0.01$) than MMI group than Vit. E alone or Se alone. As demonstrated by previous study (Ben Amara *et al.*, 2011b), showed MMI decrease SOD, and the effect of Vit. E and Se together to improve SOD levels significantly ($p<0.01$) than Vit. E (141.3% and 109% respectively) or Se (128.5% and 101.7% respectively) alone in liver and kidney.

In histological studies, the rat who treated with MMI the kidney and liver were changed (Ben Amara., 2011a; Cano-Europa., 2011). In our study, the liver rat that treated with MMI the portal spaces, hepatocytes and central area of liver lobule were clearly changed. These changes reduced to some extent in both MMI plus Vit.E and MMI plus Se, and these changes became less pronounced in MMI plus Vit.E plus Se group. In the kidney rat that treated with MMI the glomeruli and convoluted tubules were clearly changed. These changes reduced to some extent in both MMI plus Vit.E and MMI plus Se, and these changes became less pronounced in MMI plus Vit.E plus Se group.

Further studies to include female rats are needed to determine appropriate dose of antioxidant nutrients to ameliorate adverse effect from MMI treatment. We also suggest that similar studies with appropriate design could be carried out to investigate in humans the

protective effect of antioxidant supplementation of Vitamin E and Selenium on MMI toxicity. Such studies may also help in setting dietary guidelines for hyperthyroidism.

References:

1. Aboul-Enein, H.Y., and Al-Badr, A.A. (1979). Methimazole in: *Analytical Profiles of Drug Substances*, New York: Academic Press, Vol.18, pp. 351.
2. Aboul-Soud, M.A., Al-Othman, A.M., El-Desoky, G.E., Al-Othman, Z.A., Yusuf, K., Ahmad, J., and Al-Khedhairi, A.A. (2011). Hepatoprotective effects of vitamin E/selenium against malathion-induced injuries on the antioxidant status and apoptosis-related gene expression in rats, *The Journal of toxicological sciences*, 36, 285-296.
3. Ben Amara, I., Hakim, A., Troudi, A., Soudani, N., Makni, F.A., Zeghal, K.M., and Zeghal, N. (2011a). Protective effects of selenium on methimazole-induced anemia and oxidative stress in adult rats and their offspring, *Hum Exp Toxicol.*, 30, 1549-1560.
4. Ben Amara, I., Soudani, N., Troudi, A., Bouaziz, H., Boudawara, T., and Zeghal, N. (2011b). Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats, *Ecotoxicol Environ Saf*, 74, 811-819.
5. Beutler, E. (1984). *Red Cell Metabolism: Manual of Bio-chemical Methods*. 3rd Edn., Grune Stratton Inc., Orlando, FL., USA.
6. Cano-Europa, E., Blas-Valdivia, V., Franco-Colin, M., Gallardo-Casas, C.A., and Ortiz-Butron, R. (2011). Methimazole-induced hypothyroidism causes cellular damage in the spleen, heart, liver, lung and kidney, *Acta Histochem*, 113, 1-5.
7. Chen, W., Zhu, Z., Wang, C., and Chien, M. (2009). Cholestasis and Acute Cholecystitis in Hyperthyroidism Treated With Methimazole, *International Journal of Gerontology*, 3, 248-250.
8. Cooper, D. (1999). The side effects of antithyroid drugs, *Endocrinologist*, 9, 457-476.
9. El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S., and Baghdadi, H.H. (2004). Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats. *Journal of environmental science and health, Part. B, Pesticides, food contaminants, and agricultural wastes*, 39, 443-459.
10. Endreffy, E., Turi, S., Laszik, Z., Bereczki, C., and Kasa, K. (1991). The effects of vitamin E on tissue oxidation in nephrotoxic (anti-glomerular basement membrane) nephritis, *Pediatr Nephrol*, 5, 312-317.
11. Fridovich, I. (1972). Superoxide radical and superoxide dismutase, *Accounts of Chemical Research*, 5, 321-326.
12. Gallelli L, Ferraro M, Spagnuolo V, Rende P, Mauro GF, De Sarro G. (2009). Rosuvastatin-induced rhabdomyolysis probably via CYP2C9 saturation. *Drug Metabol Drug Interact* 24:83-87.
13. Kahaly, G.J., Grebe, S.K., Lupo, M.A., McDonald, N., and Sipos, J.A. (2011). Graves' disease: diagnostic and therapeutic challenges (multimedia activity), *The American journal of medicine*, 124, S2-3.
14. Koornstra, J., Kerstens, M.N., Hoving, J., Visscher, K.J., Schade, J.H., Gort, H.B., and Leemhuis, M.P. (1999). Clinical and biochemical changes following 131I therapy for hyperthyroidism in patients not pretreated with antithyroid drugs, *Neth J Med*, 55, 215-221.
15. Patra, R.C., Swarup, D., and Dwivedi, S.K. (2001). Antioxidant effects of alpha tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats, *Toxicology*, 162, 81-88.
16. Ramos-Bonner, L.S., Goldberg, T.H., Moyer, S., and Anastasopoulou, C. (2007). Methimazole-induced cholestatic jaundice in an elderly hyperthyroid patient. *Am J Geriatr Pharmacother*, 5, 236-240.
17. Stajn, A., Zikić, R., Ognjanović, B., Saicić, Z., Pavlović, S., Kostić, M., and Petrović, V. (1997). Effect of cadmium and selenium on the antioxidant defense system in rat kidneys. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*, 117, 167-172.

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