J Toxicol Pathol 2013; 26: 111–118

Original Article

Antioxidant Activity of Tocotrienol Rich Fraction Prevents Fenitrothion-induced Renal Damage in Rats

Siti Balkis Budin[1*], Kim Jit Han[1], Putri Ayu Jayusman[1], Izatus Shima Taib[1], Ahmad Rohi Ghazali[1], and Jamaludin Mohamed[1]

[1] Programme of Biomedical Science, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract: Fenitrothion (FNT) is an organophosphate compound widely used as pesticide in Malaysia. The present study aims to investigate effects of palm oil tocotrienol rich fraction (TRF) on the renal damage of FNT-treated rats. A total of 40 male Sprague Dawley rats were divided into 4 groups randomly, the control, TRF, FNT and FNT+TRF groups. FNT (20 mg/kg b.w.) and TRF (200 mg/kg b.w.) were given orally for 28 days continuously. Rats from the FNT+TRF group were supplemented with TRF 30 minutes prior to administration of FNT. Rats were sacrificed after 28 days, and the kidneys were removed for determination of oxidative stress and histological analysis. Plasma was collected for determination of blood creatinine and urea level. Statistical analysis showed that palm oil TRF has a protective effect against renal oxidative damage induced by FNT. In the FNT+TRF group, malondialdehyde and protein carbonyl levels were significantly lower, while the glutathione level as well as superoxide dismutase and catalase activities were significantly higher compared with the FNT-treated group ($p<0.05$). As for renal function, there was a markedly lower urea level ($p<0.05$) in the FNT+TRF group compared with the FNT-treated group, but there was no significant difference in creatinine level. Besides, total protein also showed no significant difference for all groups of rats ($p>0.05$). Histological evaluation also revealed that the FNT+TRF group had less glomerulus and renal tubule damage than the FNT-treated group. In conclusion, palm oil TRF was able to reduce oxidative stress and renal damage in FNT-treated rats. (DOI: 10.1293/tox.26.111; J Toxicol Pathol 2013; 26: 111–118)

Key words: organophosphate, antioxidants, renal damage, lipid peroxidation, histology

Introduction

Organophosphate pesticides (OP) have been widely used in agriculture to enhance food production and public health programs to control nuisance pests. The indiscriminate use of pesticides has indirectly threatened human health with acute and chronic poisonings[1]. OPs exert toxicity mainly through inhibition of acetylcholinesterase activity at the synapses and neuromuscular junctions, which interrupts transmission of nerve impulses. In acute OP poisonings, cholinergic effects such as excessive glandular secretion, muscle weakness, myosis and muscle cramps are observed[2]. In spite of this, oxidative stress has been proposed as an alternative mechanism of OP toxicity[3]. OPs may cause excessive production of reactive oxygen species (ROS) and result in an imbalance between the production of free radicals and cellular antioxidants[4].

Fenitrothion, or FNT [O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate], is a contact-acting organophosphate applied as a vector control agent for malaria in public health programs. The extent of FNT toxicity is dose and duration dependent. FNT has low toxicity towards mammals, as its metabolites are rapidly excreted out of the body. However, studies still showed that FNT has toxic effects on the adrenal glands, liver, kidney and other biological systems in various experimental animal models. Long-term FNT exposure increased the weight of the adrenal gland and substantially increased the glucose and corticosterone levels in the plasma of male rats[5]. Furthermore, FNT also caused liver and kidney damage as indicated by elevated serum aspartate and alanine transaminases, total protein, serum creatinine and urea[6,7].

Tocotrienol, a vitamin E analogue, is found abundantly in palm oil. It has four chemically distinct isomers, namely, alpha (α)-, beta (β)-, gamma (γ)- and delta (δ)-tocotrienol Tocotrienol is differentiated from tocopherols by its three trans double bonds in the unsaturated isoprenoid side chain. A previous study demonstrated that the tocopherol:tocotrienol ratio in palm is about 25:75[8]. Both tocopherol and tocotrienol are well known of their antioxidant properties[9]. This is due to their phenolic group (-OH) in the chromanol ring, which stabilizes free radicals by donating a hydrogen atom. How-

Received: 27 September 2012, Accepted: 4 January 2013
*Corresponding author: SB Budin (e-mail: balkis6466@yahoo.com.my)

ever, tocotrienol is more potent antioxidant than tocopherol. α-Tocotrienol and TRF were more effective in preventing lipid peroxidation in rat brain mitochondria and liver microsomes than α-tocopherol. Besides, previous study suggested that TRF reduces the diabetic-induced oxidative stress by inhibiting free radical production[10].

Hence, the aim of the current study was to investigate the antioxidant effect of TRF on FNT-induced oxidative stress and renal damage.

Materials and Methods

Animals

All animal handling procedures adhered to regulations of the Animal Ethics Committee of the Universiti Kebangsaan Malaysia. A total of 40 male Sprague Dawley rats (230–250 g b.w.) were kept in clean polypropylene cages and placed in a well-ventilated room. The animals were acclimatized for a week before the experiment. They were provided with standard rodent pellet diet and water ad libitum throughout the experiment.

Experimental design

The rats were randomly divided into four groups, each consisting of ten rats: Control, FNT, TRF and FNT+TRF. Rats in the control group were given corn oil daily, while rats in the TRF group were given 200 mg/kg body weight of TRF daily[10]. On the other hand, rats in the FNT group were given 20 mg/kg body weight of FNT daily[11]. Lastly, rats in the FNT+TRF group were given 200 mg/kg body weight of TRF followed by 20 mg/kg body weight of FNT after 30 minutes. All the treatments are given via oral gavage for 28 days consecutively. Upon completion, the rats were fasted overnight. Blood was withdrawn from the orbital sinus under light ether anesthesia for biochemical analysis. The animals were sacrificed, and the kidneys were immediately excised.

Tissue sampling

After the rats were sacrificed, kidneys were immediately removed, weighed and rinsed with chilled saline. A small portion of the kidney tissue was obtained and fixed in 10% formalin solution for histopathological examination. The remaining kidney tissue was used for biochemical analysis. Kidney tissues were minced, cut into small pieces and homogenized with 1.15% KCl at a ratio of 3 ml/g (v/w) by using Ultra-Turrax T25. The homogenate was centrifuged at 9000 g for 20 minutes at 4˚C, and the resultant supernatant was used for the determination of total protein, malondialdehyde, protein carbonyl, reduced glutathione, and activities of antioxidant enzymes.

Estimation of biochemical parameters

The extent of lipid peroxidation was estimated by measuring the concentration of malondialdehyde (MDA). In this assay, MDA reacts with thiobarbituric acid and forms pink chromogenic compounds with maximal absorbance at 532 nm. A standard graph was prepared using 1,1,3,3-tetraethoxypropane. Protein carbonyl (PC), a hallmark product of protein oxidation, was measured with spectrophotometric detection at 360 nm[12]. The reaction between 2,4-dinitrophenylhydrazine and PCO forms chromogenic protein hydrazones. Reduced glutathione (GSH) content was quantified in the kidney homogenate[13] on the basis of the reaction between 5,5-dithiobis-2-nitrobenzoic acid and GSH to form yellow colored 2-nitro-5-mercaptobenzoic acid, which can be monitored at 415 nm spectrophotometrically. Activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were estimated using colorimetric assays[14,15]. The level of creatinine and urea were estimated using commercial diagnostic kits (Biosystems, Barcelona, Spain). The Bradford assay was used to measure the protein concentration in kidney homogenate[16].

Histopathological studies

In the histological evaluation, the kidney sections were fixed in 10% formalin, dehydrated by passing through graded series of alcohols and water and cleared in xylene. The processed tissues were embedded in paraffin, cut into sections 3–5-µm thick by using a microtome and stained with hematoxylin and eosin (H&E) dye. Mounted slides were observed and photographed under a light microscope.

Statistical analysis

All numerical data were expressed as means ± SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and post hoc test. Values were considered statistically significant when $p < 0.05$.

Results

Effect of TRF on renal oxidative damage

As depicted in Fig. 1, the MDA level in kidney homogenate of the FNT-treated group was significantly higher ($p<0.05$) as compared with the control and TRF group. TRF had a protective effect against oxidative stress as shown by the lower ($p<0.05$) MDA level in the FNT+TRF group as compared with the FNT-treated group alone. Similarly, the PC level of the FNT-treated group also was significantly higher than those of the control and TRF groups. The FNT+TRF group had significantly lower PC levels as compared with the FNT-treated group alone (Fig. 2).

Effect of TRF on renal antioxidants status

As for antioxidant levels in the rat kidney, the GSH level was significantly lower ($p<0.05$) in the FNT-treated group alone compared with the control group. However, the GSH level in the FNT+TRF group was remarkably elevated, suggesting the potential in restoring GSH content in FNT-treated rats (Fig. 3). SOD and CAT activities were significantly lower ($p<0.05$) in the FNT group alone compared with the control and TRF groups. On the other hand, the FNT+TRF group had significantly higher SOD (Fig. 4) and CAT (Fig. 5) activities than those of the FNT-treated group.

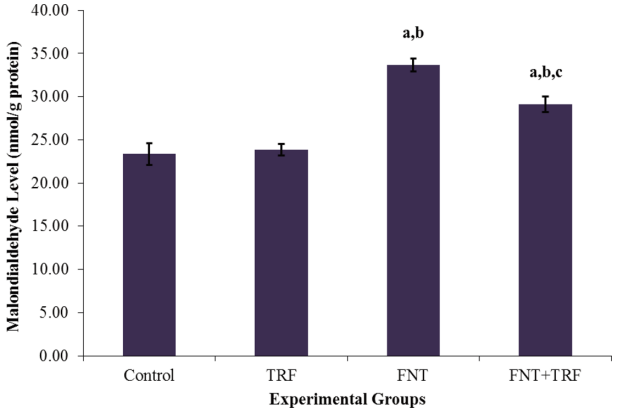

Fig. 1. Level of MDA in renal homogenate by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

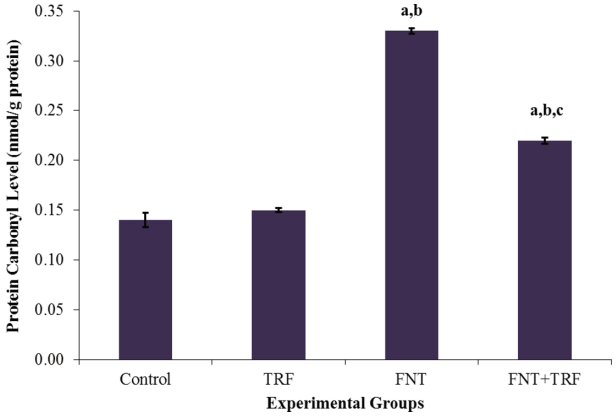

Fig. 2. Level of PC in renal homogenate by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

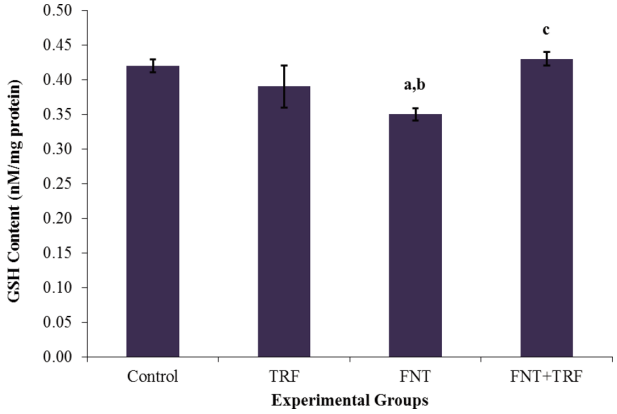

Fig. 3. Level of GSH in renal homogenate by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

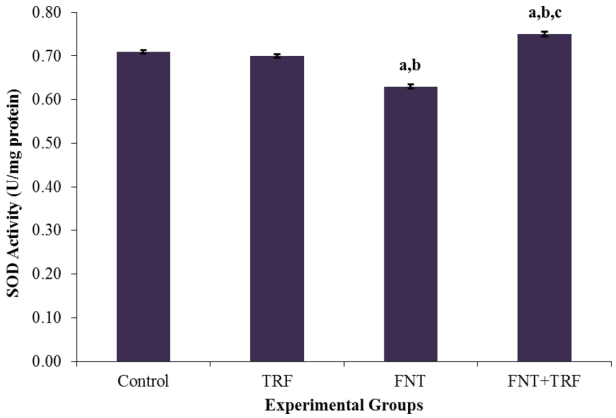

Fig. 4. Activity of SOD in renal homogenate by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

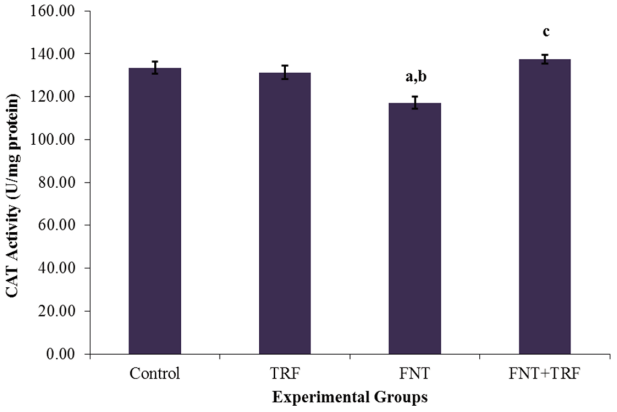

Fig. 5. Activity of CAT in renal homogenate by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

Effect of TRF on renal function parameters

Figure 6 depicts the mean creatinine content of each experimental group. It was revealed that no significant difference ($p>0.05$) was present among the groups. However, plasma urea in the FNT-treated group was significantly higher ($p<0.05$) as compared with the control and TRF groups respectively. However, a significantly lower ($p<0.05$) level of urea was recorded in plasma of the FNT+TRF group as compared with the FNT-treated group alone (Fig. 7).

Effect of TRF on renal histology

Figures 8a to 11b show the renal histology of each experimental group. The histological examination of kidneys sections of the control (Fig. 8a and 8b) and the TRF groups (Fig. 9a and 9b) revealed normal renal corpuscles consisting of a Bowman's capsule with an outer layer of simple squamous epithelium and inner layer of flattened epithelial cells.

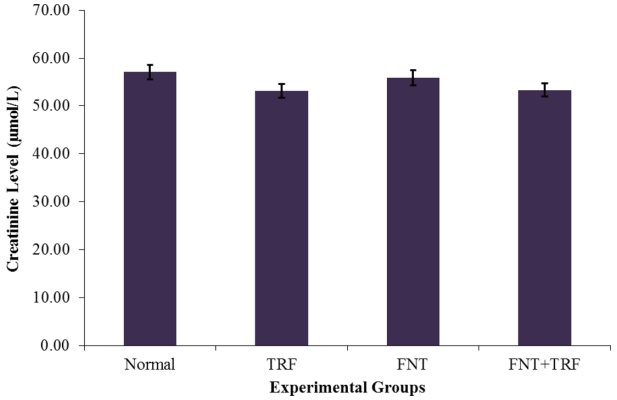

Fig. 6. Creatinine level in blood plasma by experimental groups (mean ± SEM).

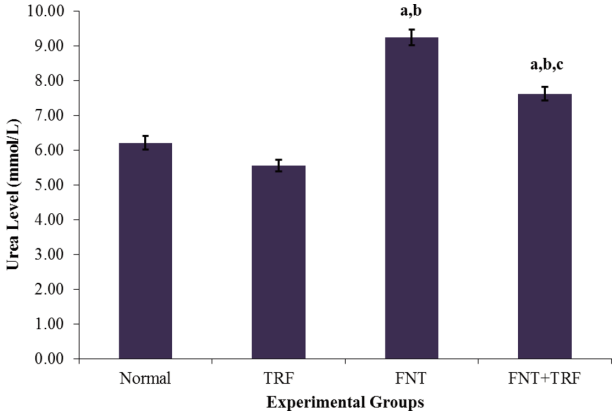

Fig. 7. Urea level in blood plasma by experimental groups (mean ± SEM). [a] Significantly different from control group ($p<0.05$). [b] Significantly different from TRF group ($p<0.05$). [c] Significantly different from FNT group ($p<0.05$).

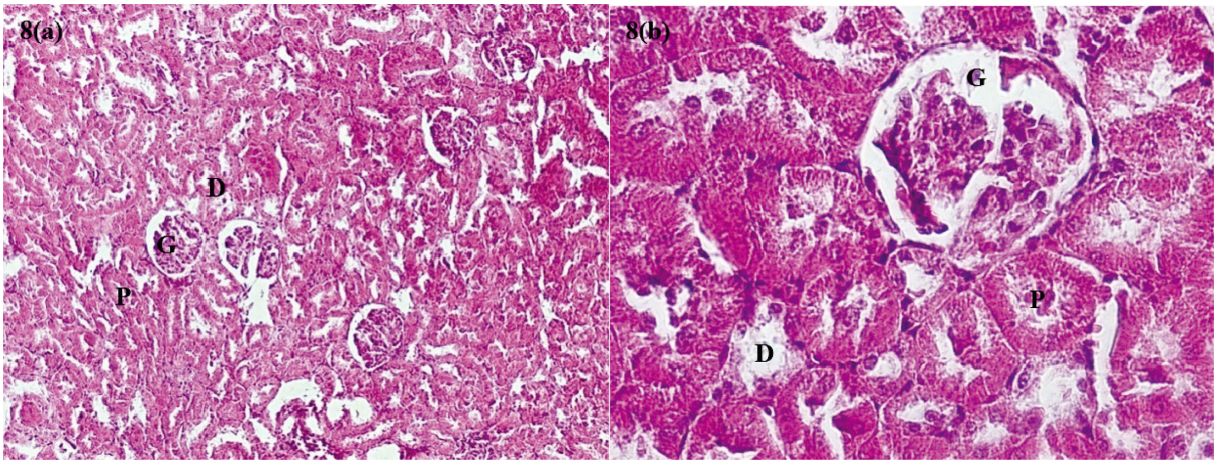

Fig. 8. (a) Kidney of a control, untreated rat showing normal renal corpuscles with glomeruli (G) and sections of proximal (P) and distal convoluted tubules (D) with a cuboidal epithelial lining (×100). (b) Kidney of a control, untreated rat showing a normal cortex with normal corpuscle and tubules (×400).

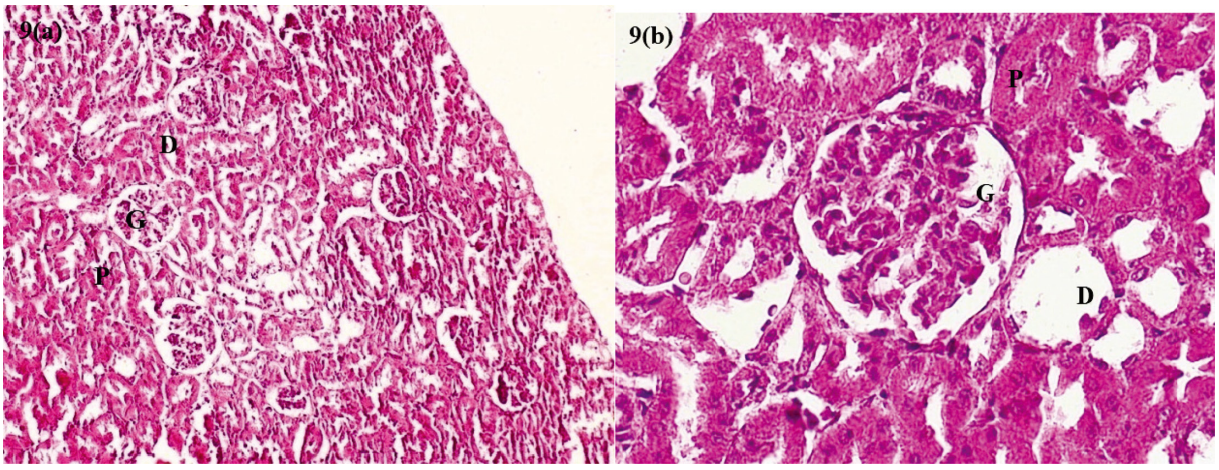

Fig. 9. (a) Kidney of a rat treated with palm oil TRF showing normal renal corpuscles with glomeruli (G) and proximal (P) and distal convoluted tubules (D) with a cuboidal epithelial lining (×100) (b) Kidney of a rat treated with palm oil TRF showing a normal corpuscle and tubules (×400).

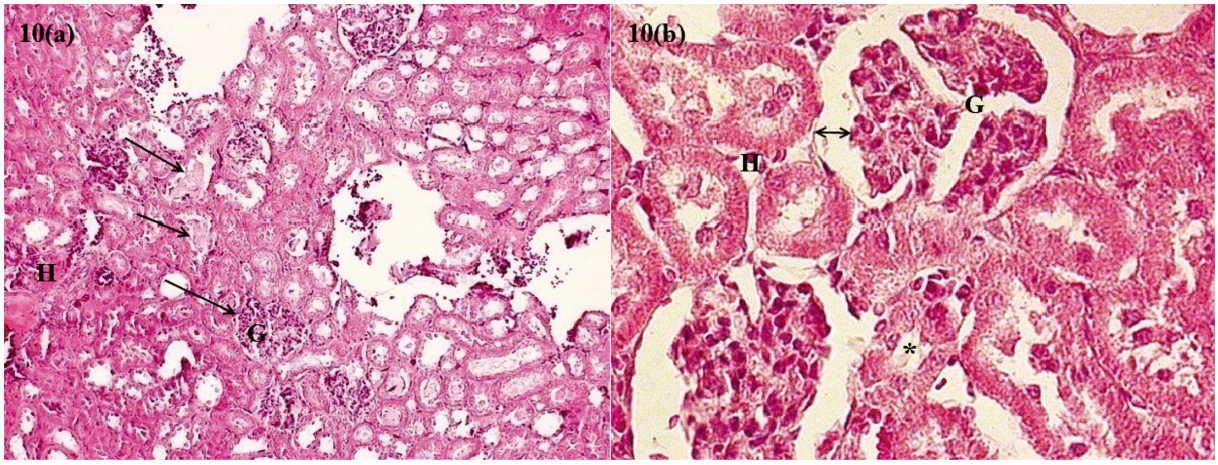

Fig. 10. (a) Kidney of an FNT-treated rat showing distortion of the renal architecture. There are small areas of congestion and hemorrhage (H) in the cortex. Arrows indicate the presence of protein cast in the renal tubule (×100). (b) Kidney of an FNT-treated rat showing a mild shrunken glomerulus (←→). Mild degenerative changes in tubular epithelial lining (*) and hemorrhage (H) between tubules (×400).

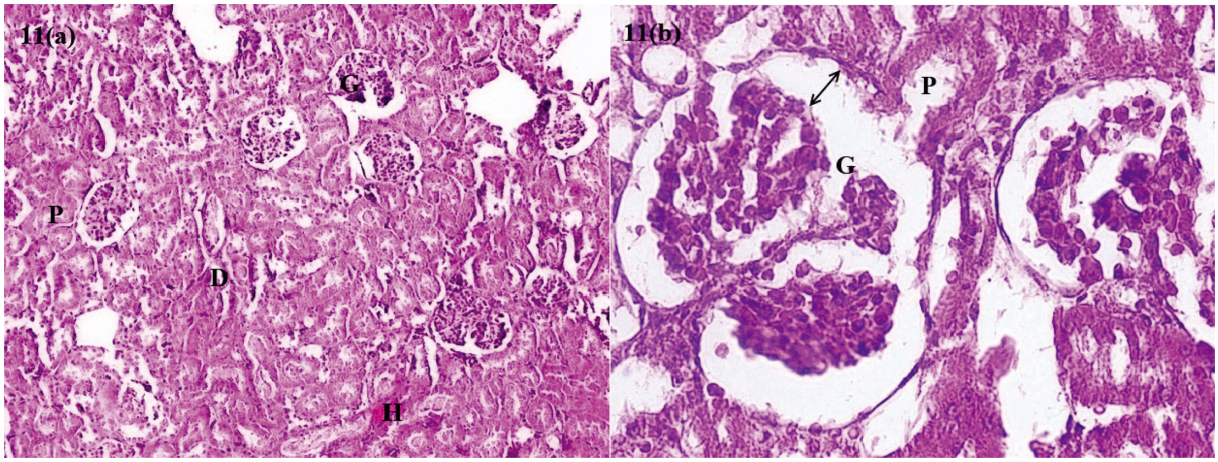

Fig. 11. (a) Kidney of an FNT+TRF-treated rat showing marked improvement in renal architecture, but still a few areas of hemorrhage (H) between them (×100). (b) Kidney of an FNT+TRF-treated rat that was comparable to that in the control group. Only mild shrunken glomeruli (←→) were observed (×400)..

The corpuscle was surrounded by a tuft of blood capillaries, a glomerulus. Sections of the proximal and distal convoluted tubules could be seen with their cuboidal lining. The examination of the kidney sections from the FNT-treated group (Fig. 10a and 10b) showed tubulointerstitial changes. In the FNT alone group, simple dilatation of Bowman's capsule was seen. Severe hemorrhage was seen in the cortex region. Renal tubules were swollen and distorted with necrosis affecting the epithelial lining. There were visible changes in morphology such as widening between parietal and visceral layers of Bowman's capsules. In addition, the renal cortex showed mild congestion and hemorrhage. Furthermore, a little mononuclear cell infiltration was also noted in this group. On the other hand, the FNT+TRF group stood out due to a partial reduction in kidney damage as compared with the group treated with FNT, and most of the sections in the FNT+TRF group were comparable to the control group. Administration of TRF was unable to fully restore glomeruli and renal tubules, but hemorrhage was inhibited in the renal cortex. Partial necrosis affected glomeruli in the FNT+TRF group. Only the kidney cortex showed slightly shrunken glomerulus, swelling of the epithelial tubular lining with a few areas of hemorrhage between them (Fig. 11a and 11b).

Discussion

Extensive use of organophosphate insecticides can be detrimental to mammals. Apart from acute cholinergic poisoning, organophosphate intoxication also induces oxidative stress with increased generation of reactive oxygen species (ROS). In the current study, the protective role of palm

oil TRF against fenitrothion-induced oxidative stress and damage in the rat kidney was demonstrated. TRF and FNT may have interaction when administered together. FNT is an organophosphate compound that has the ability to generate free radicals spontaneously. When TRF is ingested, its blood concentration is affected, as it acts to scavenge free radicals generated by FNT. Therefore, the concentrations of both TRF and FNT are lowered in the blood and only the remaining portion could be absorbed into tissues.

Lipid peroxidation, a ROS-mediated process, has been demonstrated to be involved in the pathogenesis of numerous kidney diseases. The major oxidation product of peroxidized polyunsaturated fatty acid in the cell membrane is malondialdehyde (MDA). Thus, accumulating MDA in tissues acts as an indicator for lipid peroxidation[17]. In the present study, the renal MDA level was significantly high in the FNT-treated rats. This result is in agreement with previous studies as reported for other Ops, e.g., FNT in hepatocytes[18], diazinon in the myocardium[19] and methyl parathion in the kidney[20]. Protein carbonyl (PC) is another biomarker that indicates protein oxidation. The elevation of PC content is due to ROS-mediated protein oxidation or secondary modifications due to the presence of stable adduction compounds, reactive aldehydic end products of lipid peroxidation and amino acid side chains that lead to structural alteration and functional inactivation of enzyme proteins[21]. In the current study, FNT-intoxicated rats also had a prominent elevation in the level of renal PC.

Supplementation of FNT-treated rats with palm oil TRF markedly reduced MDA and PC levels in renal tissues when compared with FNT-treated rats alone. TRF acts as a potential antioxidant in reducing lipid peroxidation and protein oxidation after FNT toxicity. Tocotrienols in palm oil are lipophilic antioxidants that can be localized in the cellular membranes to protect against lipid peroxidation. They scavenge and quench ROS in the cells via a chain breaking mechanism that neutralizes peroxyl and alkoxyl radicals generated during lipid peroxidation[9]. In addition, α-tocotrienol has a 40-60 times higher antioxidant efficacy to protect against Fe (II) +NADPH-induced lipid peroxidation than α-tocopherol[22]. TRF can prevent oxidative-mediated modification of protein molecules through inhibition of carboxymerhyl lysine[23] formation by albumin glycation.

Reduced glutathione (GSH) is a nonenzymatic radical scavenger that eliminates free radicals generated from oxidative metabolism[24]. It detoxifies reactive metabolites through conjugation with its sulfhydryl group. In this study, a significant depletion of the GSH level in the kidney after FNT exposure was observed. A low renal GSH content is probably due to excessive involvement of GSH in neutralization of peroxide radicals[25]. On the other hand, SOD and CAT are considered primary enzymatic antioxidants that protect cells from ROS toxicity and lipid peroxidation. SOD catalyzes the dismutation of superoxide radicals to hydrogen peroxide, while CAT cleaves this hydrogen peroxide into molecules of water and oxygen[26]. It is known that xenobiotics can induce superoxide radical production in mitochondria[27], and the amount of superoxide radical can reach a critical level if SOD is significantly inhibited. The superoxide radical is a potent inhibitor of CAT[28]. In addition, oxidative modification of enzymes may be present, as indicated by an increased level of PC. These reasons explain low SOD and CAT activity in FNT-intoxicated rats.

Interestingly, supplementation with TRF was able to restore the GSH level, suggesting that TRF reduces the concentration of free radicals in the kidney. Due to a lower level of oxidative stress, the level of GSH is maintained in a normal state to counteract the endogenous action of free radicals. At the same time, supplementation of the FNT-treated rats with TRF for 28 days was shown to improve SOD and CAT activities. In the FNT+TRF group, the antioxidant effect of TRF was overwhelmed, resulting in the SOD and CAT activities being higher than those in the normal group. Due to its antioxidant traits, TRF could enhance the endogenous antioxidant defence system in biological systems. The current results were similar to those of a previous study in which TRF supplementation increased SOD activities in diabetes-induced oxidative stress[29].

Creatinine and urea are biomarkers used to evaluate kidney function. There should more than 70% nonfunctional nephrons before any obvious elevations in plasma urea or creatinine level can be observed. In the present study, it was demonstrated that the rats treated with FNT exhibited a significant increase in plasma urea but a nonsignificant reduction in plasma creatinine when compared with control rats. The creatinine outcome is paradoxical to previous studies in which there was an increase in serum creatinine when the rats were treated with diazinon (an OP), but the urea result is consistent with another study, in which blood urea nitrogen (BUN) was elevated[30]. This is possibly due to insufficiency of dosage and duration of FNT treatment which only leads to mild renal damage instead of renal failure. Thus, the kidneys were still able to excrete plasma creatinine in urine. The mild reduction in plasma creatinine level may be attributed to a decrease in the muscle mass of FNT-treated rats[31]. However, there is a significant increase in plasma urea in FNT-treated rats. This suggests that 28-day FNT treatment (20 mg/kg) caused mild impairment in kidney function, which could probably be due to oxidative damage. Supplementation of TRF at 200 mg/kg for 28 consecutive days was able to decrease the plasma urea level but not the creatinine level in FNT-treated rats. The decline in plasma urea is possibly due to the antioxidant effect of TRF in protecting against FNT-induced oxidative damage of kidneys. As reported previously, pretreatment with TRF and α-tocopherol in iron-induced rats significantly reduces the serum creatinine and BUN levels and simultaneously decreases lipid peroxidation and restores normal levels of antioxidant[32]. Furthermore, TRF supplementation reduces the level of BUN in heavy metal intoxication. This suggests that palm oil TRF has a significant nephroprotective effect against oxidative stress[33].

These biochemical findings are in close agreement with the histological study. In the current study, only mild

structural abnormalities were observed in the renal cortex after FNT administration. Induction of FNT at 20 mg/kg for 28 consecutive days demonstrated mild degeneration changes of renal architecture. Besides, there was congestion and hemorrhage confined only to a small area of the renal cortex. In addition, mild shrunken tufts of capillaries (glomeruli) were noticed in FNT-treated rats. From the microscopic examination, it can be noted that treatment with FNT for 28 days is insufficient to cause extensive histological damage in the kidneys. Supplementation of palm oil TRF lowered the severity of renal damage as compared with the group treated with FNT alone. Histological observation indicated marked ameliorative improvement in renal architecture with only a few shrunken glomeruli, and a small area of haemorrhage between them was discovered. This observation was similar to an earlier study in which treatment with vitamins E and C resulted in less significant histopathological damage in the liver of ethion-treated rats[34].

To summarize, supplementation with 200 mg/kg of palm oil TRF was found to have a beneficial effect in attenuating FNT-induced oxidative damage by reducing oxidative stress and improving the antioxidant level in FNT-treated rats. Meanwhile, oral administration of TRF was able to markedly improve renal function by ameliorating oxidative stress-induced renal damage in FNT-treated rats.

Acknowledgements: We would like to acknowledge Mr Anand Ramalingam and Mdm Azmira Mohd Sedik for their assistance and technical support. Financial support was provided by grant (UKM-NN-03-FRGS0042-2010) from Ministry of Higher Education (MOHE).

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