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Ameliorative Effect of Palm Oil Tocotrienol Rich Fraction on Brain Oxidative Stress in Fenitrothion-administered Rats

(Kesan Pemulihan Pecahan Kaya Tokotrienol Minyak Kelapa Sawit terhadap Tekanan Oksidatif Otak Tikus Diadministrasi Fenitrotion)

SITI BALKIS BUDIN*, IZATUS SHIMA TAIB, PUTRI AYU JAYUSMAN, HUI HUI CHIANG, ANAND RAMALINGAM, AHMAD ROHI GHAZALI & JAMALUDIN MOHAMED

ABSTRACT

Fenitrothion (FNT) usage has received much attention for its potential to promote free radicals generation and interfere with antioxidant defense system. The aim of the present study was to investigate the effect of palm oil tocotrienol rich fraction (TRF) supplementation on oxidative stress and histological changes in rat brain induced by FNT. A total of 32 male Sprague Dawley rats divided into four groups: control group which received corn oil; TRF group was received palm oil TRF (200 mg/kg bw); FNT group administered with FNT (20 mg/kg bw) and TRF+FNT group pretreated with palm oil TRF (200 mg/kg bw) 30 min prior to administration of FNT (20 mg/kg bw). FNT and TRF were dissolved in corn oil and all supplementations were given by oral gavage once daily for 28 days. After four weeks of supplementation, TRF+FNT rats had significantly lower malondialdehyde (MDA) content and superoxide dismutase (SOD) activity but higher reduced glutathione (GSH) level and total protein level compared to FNT rats ($p < 0.05$). However, protein carbonyl (PC) level was insignificantly lower for TRF+FNT group compared to FNT group. In conclusion, this study suggested that palm oil TRF was effective in preventing brain damage in rats.

Keywords: Fenitrothion; oxidative damage; palm oil tocotrienol rich fraction

ABSTRAK

Kegunaan fenitrothion (FNT) telah mendapat perhatian ramai kerana insektisida ini berupaya untuk mengaruh penjanaan radikal bebas dan mengganggu sistem pertahanan antioksidan. Tujuan kajian ini adalah untuk mengetahui kesan pecahan kaya tokotrienol (TRF) minyak sawit terhadap tekanan oksidatif dan perubahan histologi otak tikus teraruh FNT. Tiga puluh dua ekor tikus Sprague Dawley jantan telah dibahagikan kepada empat kumpulan: kumpulan kawalan yang menerima minyak jagung; kumpulan TRF yang menerima TRF (200 mg/kg bw); kumpulan FNT yang diadministrasi FNT (20 mg/kg bw) dan kumpulan TRF+FNT yang diberi TRF (200 mg/kg bw) 30 min selepas diadministrasi FNT (20 mg/kg bw). FNT dan TRF telah dilarutkan dalam minyak jagung dan semua suplemen telah diberi secara oral untuk 28 hari. Selepas empat minggu suplementasi, tikus TRF+FNT menunjukkan penurunan aras malonaldehid (MDA) dan aktiviti superoksida dismutase (SOD) tetapi aras glutathion terturun (GSH) serta protein jumlah lebih tinggi berbanding tikus FNT ($p < 0.05$). Namun, aras karbonil protein (PC) kumpulan TRF+FNT adalah rendah berbanding kumpulan FNT secara tidak signifikan. Kesimpulannya, kajian ini mencadangkan bahawa TRF minyak sawit dapat mengurangkan tekanan oksidatif dan mengelakkan kerosakan otak tikus aruhan FNT secara berkesan.

Kata kunci: Fenitrotion; kerosakan oksidatif; pecahan kaya tokotrienol minyak sawit

INTRODUCTION

Fenitrothion (O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) is an organophosphate belonging to Class II insecticide with low mammalian toxicity and extensively used to control insect pests and mites on cotton, orchard fruits, rice, cereals and vegetables (Uygun et al. 2005). FNT is a contact-acting insecticide and applied as a vector control agent for malaria in public health programs. FNT tends to precipitate in watery system where the activity of microorganisms is active (Xavier et al. 2004). Dermal, oral and inhalation exposure to FNT inhibits the activity of acetylcholinesterase (AChE) irreversibly in blood plasma and brain in mammals, resulting accumulation

of acetylcholine. Subsequently, acetylcholine activates muscarinic and nicotinic receptors extensively and causes cholinergic symptoms include myosis, lacrimation, salivation, increased urination, diarrhea and diaphoresis (Kwong 2002).

Besides acting as AChE inhibitor, the toxic effects of FNT probably occurred through generation of reactive oxygen species (ROS) causing oxidative damage to membranous cell components (Goel et al. 2005). Oxidative stress occurred when there is excessive generation of free radical and loss of antioxidant ability to inactivate them. This will eventually leads to oxidative damage to lipids, proteins, carbohydrates and nucleic acid. A study showed

that oxidative damage and cytotoxic effect on rat brain can be induced by high dose of FNT plus lambda-cyhalothrin for a long period of exposure (El-Demerdash 2011).

Brain is highly susceptible to oxidative damage due to its high polyunsaturated lipid and ferum content, high oxygen consumption, formed by largely non-mitotic cells and lack of antioxidant defense system such as catalase (Contreras et al. 2000). Oxidative modification changes the functioning lipids and proteins, promoting formation of highly reactive products which can further modify these cellular targets, as well as lack of antioxidant protection in brain to neutralize ROS. Alteration of lipid and protein constituents leads to loss of cell function and viability as well as cell-to-cell communication breakdown. Moreover, oxidative stress trigger cascade of ROS-dependent signaling that activates mechanism of apoptosis and cell necrosis in brain (Aksenova et al. 2005).

Discovery of the neuroprotective effects of antioxidants, enzymes and natural products are used to suppress oxidative damage in various organs induced by OP compounds. Vitamin E was recognised as a lipid soluble antioxidant which acts as chain breaking agent and ROS scavenger that prevent free radical mediated peroxidative tissue damage. Tocotrienol rich fraction (TRF) from palm oil sources is a rich natural source of vitamin E, which consists of 70% tocotrienol and 30% tocopherol. Tocotrienol and tocopherol had similar chemical structure and gave synergistic effect when co-administered but a slightly deviation in their antioxidant activity. TRF is an excellent antioxidant and it has been effectively used as a nutritional supplement due to its potential therapeutic benefits (Therriault et al. 1999).

The present study was undertaken to investigate the ameliorative effects of palm oil TRF on oxidative stress in rat brain induced by FNT. It can be beneficial by improving neurological conditions in insecticide-induced poisoning.

MATERIALS AND METHODS

CHEMICALS

All chemicals used were of analytical grade. Fenitrothion (99%) was purchased from Supelco, Sigma-Aldrich, USA. Palm oil tocotrienol rich fraction branded as Gold Tri-E 70 was obtained from Sime Darby, Malaysia.

ANIMALS

Male *Sprague Dawley* rats (230-250 g) were obtained from Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. The study was approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). The rats were housed in an experimental laboratory for 1 week prior to commencement of the experiment. Two animals were housed in each polypropylene cages at ambient temperature and fed on standard rat chow and water *ad libitum*.

EXPERIMENTAL DETAILS

Thirty-two male *Sprague Dawley* rats were weighed and randomly divided into four groups: Control group, TRF group, FNT group and TRF+FNT group. Control group and TRF group received corn oil and palm oil TRF (200 mg/kg bw) (Budin et al. 2009), respectively. FNT group was administered with FNT (20 mg/kg bw) while TRF+FNT group pretreated with palm oil TRF (200 mg/kg bw), 30 min prior to administration of FNT as adapted from Elhalwagy et al. (2008). The regimens were given orally by gavage once daily for a period of 28 days.

SAMPLE COLLECTION

After four weeks of supplementation, the animals were anesthetized by diethyl ether and blood samples were collected. After the animals were sacrificed, brain was removed immediately, weighted and washed using chilled saline solution. A portion of cerebellum was immersed in 10% formalin for histology purpose. Brain tissue were minced and homogenized (10% w/v) in ice-cold 1.15% KCl. The homogenate was centrifuged at 8000 rpm for 20 min at 4°C and stored at -40°C until analysis.

ACETYLCHOLINESTERASE ACTIVITY IN BRAIN

Acetylcholinesterase enzyme activity was determined according to the method of Ellman et al. (1961). Cholinesterase reagent set (PTC) from Teco Diagnostics was used in this study based on Ellman principle. AChE activity was measured spectrophotometrically at 405 nm and expressed as U/L.

OXIDATIVE DAMAGE MARKERS IN BRAIN

Lipid peroxidation was determined via measurement of the end product of lipid peroxidation, malonaldehyde (MDA) spectrophotometrically by using the method of Hunter and Jamaludin (1986). MDA measurement was based on colorimetric method where the chromogen produced by the reaction of thiobarbituric acid (TBA) and MDA molecules can be measured at the wavelength of 535 nm. 1,1,3,3-tetraethoxypropane was used as standard and MDA values was expressed as nmoles/L. The final concentration of MDA is in unit nmoles/mg protein. Protein carbonyl (PC) concentration in brain tissue was determined based on the method of Levine et al. (1990). PC content was measured spectrophotometrically at 360 nm and expressed in nmoles/mg protein.

ANTIOXIDANT ACTIVITY, REDUCED GLUTATHIONE AND TOTAL PROTEIN LEVEL

The antioxidant enzyme, superoxide dismutase (SOD) was assayed according to Beyer and Fridovich (1987). The assay involves the reduction of nitroblue tetrazolium (NBT) by superoxide radical and formed a blue formazan dye. The absorbance was measured spectrophotometrically at 560 nm. Units of SOD activity is defined as the enzymes required

for 50% reduction of NBT and the activity was expressed as unit enzyme (U)/mg protein. Reduced glutathione (GSH) content was quantified using Ellman's reagent (1959). The method is used to measure the level of reduced glutathione conjugates with DTNB resulting a yellow conjugate GSH-DTNB. The reaction was monitored at 412 nm and the amount of GSH was expressed as mmoles/mg protein.

HISTOPATHOLOGICAL STUDIES

Histological observation of cerebellum tissue was performed by using Hematoxylin and Eosin (H&E) staining method.

STATISTICAL ANALYSIS

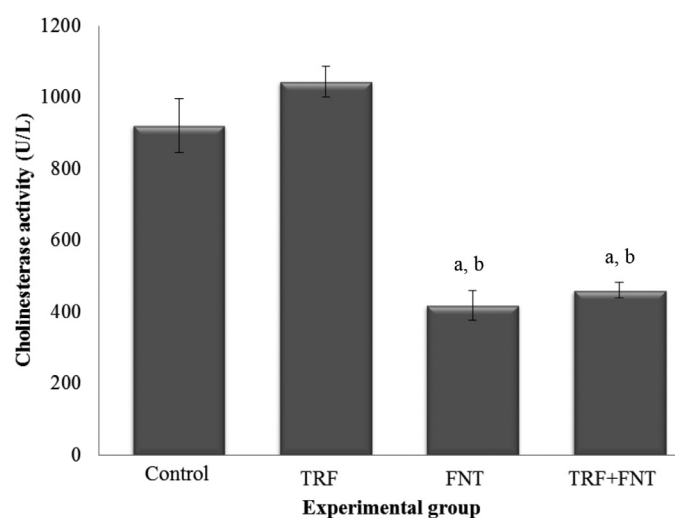
All values were expressed as mean \pm standard error of mean (SEM). Differences in experimental groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or Games Howell test. Statistical significance of the difference in value of different groups was calculated by (F) test at 5% significance level.

RESULTS

As shown in Figure 1, the administration of FNT to rats caused a significant lower ($p < 0.05$) activity of brain AChE compared with control and TRF group, the same result was obtained from TRF+FNT group. Pretreatment with palm oil TRF in FNT-administered rats showed no significant difference compared with FNT group.

Table 1 shows the effect of different treatments on brain oxidative damage and antioxidant status. The results showed that there was a significant higher ($p < 0.05$) MDA concentration in FNT group compared with control and the TRF group. Pretreatment with palm oil TRF prior to FNT-administered rats significantly lowered ($p < 0.05$) MDA concentration compared with the FNT group. The administration of FNT alone showed a significantly higher ($p < 0.05$) level of PC in rat brain compared with control and the TRF group. PC level was lower in TRF+FNT group as compared with FNT group.

Apart from that, the results showed significantly higher ($p < 0.05$) SOD activity in FNT group compared with control and the TRF group. Pretreatment with palm oil TRF prior to FNT administration (TRF+FNT) significant



^a $p < 0.05$ compared with corresponding value of Control. ^b $p < 0.05$ compared with corresponding value of TRF

FIGURE 1. Activity of acetylcholinesterase (AChE) in brain homogenate. The data are expressed in mean \pm SEM

TABLE 1. Oxidative damage and antioxidant status in brain homogenate

| Parameter | Control | TRF | FNT | TRF+FNT |
|------------------------|-------------------|-------------------|----------------------------------|----------------------------------|
| MDA (nmol/mg protein) | 12.31 \pm 1.05 | 8.81 \pm 1.52 | 24.30 \pm 1.58 ^{a, b} | 14.98 \pm 1.22 ^{b, c} |
| PC (nmol/mg protein) | 0.72 \pm 0.06 | 0.57 \pm 0.05 | 1.13 \pm 0.10 ^{a, b} | 0.88 \pm 0.07 ^{b, c} |
| SOD (U/min/mg protein) | 0.92 \pm 0.03 | 0.84 \pm 0.06 | 1.25 \pm 0.07 ^{a, b} | 0.95 \pm 0.05 ^c |
| GSH (mmol/mg protein) | 0.081 \pm 0.002 | 0.076 \pm 0.001 | 0.071 \pm 0.002 ^a | 0.080 \pm 0.002 ^c |

The data are expressed in mean \pm SEM. ^a $p < 0.05$ compared with corresponding value of Control. ^b $p < 0.05$ compared with corresponding value of TRF. ^c $p < 0.05$ compared with corresponding value of FNT

lowered ($p<0.05$) the activity of SOD in brain compared with FNT group. Besides, the administration of FNT alone significantly lowered ($p<0.05$) GSH content in brain homogenate compared with control. However, GSH content in TRF+FNT group was significantly higher compared with the FNT group ($p<0.05$).

Normal morphology of cerebellum with orderly arrangement of purkinje cells which located in between of molecular and granule cell layers were observed in all groups (Figure 2). The administration of FNT failed to induce any morphological changes.

DISCUSSION

Overwhelming application of insecticide leads to serious pollution and health problems to human. Exposure to OP insecticide contaminates agriculture products and drinking water source (Xavier et al. 2004). OP is a well-established AChE inhibitor, thus marked decline in this enzyme activity acts as an indicator OP poisoning. The present study showed that FNT-induced oxidative stress decreases AChE activity. However, oral supplementation of palm oil TRF had shown no effects against FNT-induced AChE inhibition in rats. This suggested that supplementation of palm oil TRF might not play acts as direct antidote for OP-induced inhibition of AChE enzymes.

The present study also demonstrated that oxidative stress induced by FNT leads to increase in MDA level consistent with previous studies (El-Demerdash 2011;

Lukaszewics-Hussain 2008). As a potent pro-oxidant, FNT generates free radicals excessively, targeting lipid membranes. Thus, more lipid peroxidation occurs as indicated by MDA level. TRF supplementation significantly lowered lipid peroxidation in brain. Accordingly, Kamat and Devasagayam (1995) has demonstrated that palm oil TRF is capable to reduce lipid peroxidation in brain mitochondria *in vitro*. α -Tocotrienols in palm oil TRF exerts higher antioxidant potency due to its polyphenolic lipids that enable it to move freely and react with lipid radicals in the bilayer membrane.

PC is the oxidized form of protein which regularly found when protein oxidation present. The present study showed that a significant increase in PC induced by oxidative stress in FNT rats and the findings are in accordance with previous study (Amara et al. 2011). Protein oxidation may due to the overproduction of ROS which will actively bind to the functional group of protein and forms stable protein carbonyl. Elevation in protein carbonyl level may due to the reduction of proteasome ability to degrade oxidized proteins. In this study, pre-treatment with palm TRF was able to reduce PC content in brain tissue. It appears to minimize and lower PC formation possibly through its antioxidant activity.

Palm oil TRF is well known as a lipid soluble antioxidant which exerts the ability of scavenging peroxy radical effectively and acts as a chain-breaking agent (Yoshida et al. 2007). The combination of tocotrienol and tocopherol is naturally found in palm oil TRF with a higher

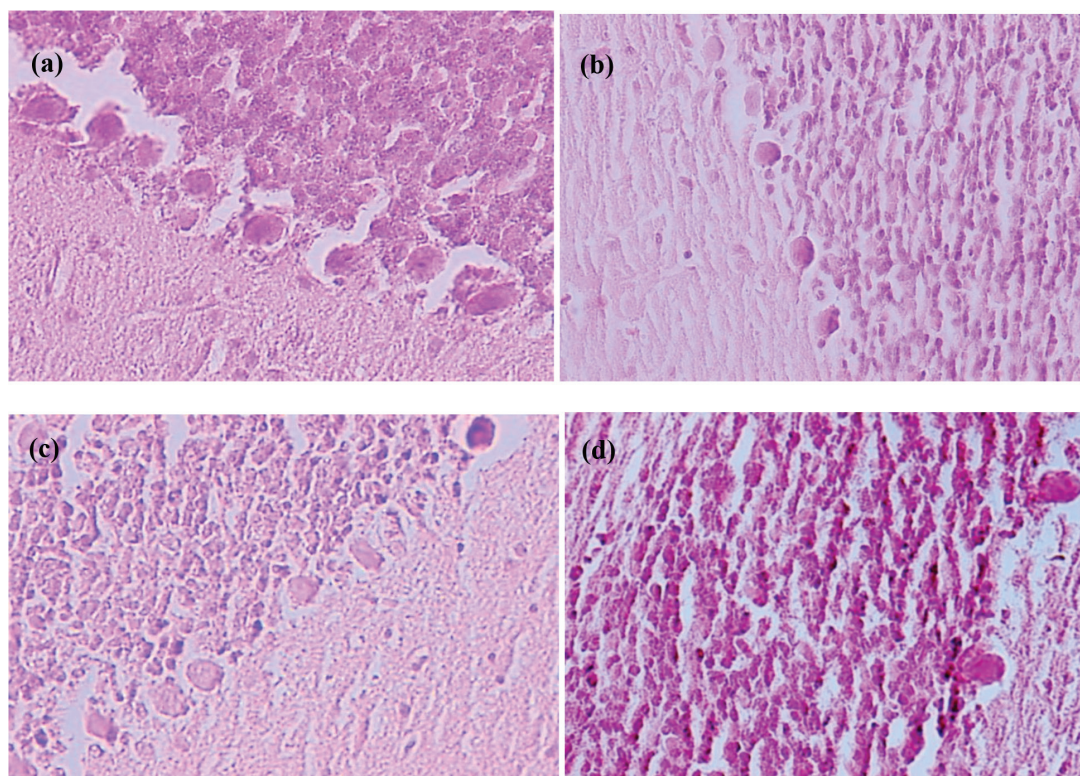


FIGURE 2. Photographs of cerebellum (H&E) in (a) control, (b) TRF, (c) FNT and (d) TRF+FNT groups. No difference was seen among groups

content of tocotrienol. They act synergistically and enable a more effective lipoprotein transport to the target organs. The antioxidant effect of α -tocotrienol is more potent compared with α -tocopherol. This is due to the presence of isoprenoid side chain and methylated chromanol ring in α -tocotrienol enable it to draw closer membrane surface, orderly distributed and easily integrated into cell membrane (Frank et al. 2012).

The increased SOD activities and reduced GSH content in brain described that the presence of oxidative stress in rat brain after FNT exposure. Increase in brain SOD activity is mainly due to excessive superoxide anion production results in lipid peroxidation (Saulsbury et al. 2009). Elevation in enzymatic antioxidant is followed by reduction in GSH content due to the ROS generation (Amara et al. 2011). Merad-Boudia et al. (1998) reported that depletion in GSH content in brain will disturb complex activity in electron transport chain and increase ROS generation. Palm oil TRF supplementation showed no significant changes in SOD activity in brain, similar to previous studies (Shirpoor et al. 2009; Tiwari et al. 2009). Meanwhile, supplementation of palm oil TRF able to attenuate the reduction of GSH level and it might be due to decrease in oxidative stress as shown in this study. Supplementation with palm oil TRF replenished GSH level in diabetes rats through its effective antioxidant activity against oxidative stress (Budin et al. 2011). The protective mechanism of TRF supplementation is suggested that its antioxidant properties to ameliorate lipid peroxidation and oxidative stress in brain.

CONCLUSION

In summary, the present study clearly demonstrated that supplementation with palm oil TRF was effective to reduce oxidative stress and damage in brain induced by FNT through reducing lipid peroxidation and improve antioxidant enzyme activities.

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Program 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

*Corresponding author; email: balkis6466@yahoo.com.my

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