

Red fruit oil supplementation fails to prevent oxidative stress in rats

Maria Dara Novi Handayani*, Parwati Abadi Soekarno**, and Septelia Inawati Wanandi**

ABSTRACT

*Department of Biochemistry,
Faculty of Medicine,
Atma Jaya Catholic University
**Department of Molecular
Biology and Biochemistry,
Faculty of Medicine,
University of Indonesia

Correspondence

dr. Maria Dara Novi
Handayani, M.Biomed
Department of Biochemistry,
Faculty of Medicine
Atma Jaya Catholic
University
Pluit Raya No.2
Jakarta Utara, 14440
Jakarta Indonesia
Phone: +6221-6694366
ext. 203
Email:
dara.sudarya@gmail.com

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BACKGROUND

Red fruit oil (RFO) is popular in Indonesia especially in Papua. It is consumed by people for maintaining health and to treat diseases. Red fruit (*Pandanus conoideus* Lam) oil is reported to contain the antioxidants α -tocopherol and β -carotene. The aim of this study was to confirm the protective effect of RFO against decreased glutathione levels in the plasma and liver of rats induced by 2N-(2-fluorenyl) acetamide (2-FAA).

METHODS

Wistar male rats weighing 200 ± 20 g were randomly assigned into a control group (receiving distilled water only) and three intervention groups, designated RFO, FAA, and RFO-FAA (n=6 rats per group). RFO was given RFO 10 μ l/g/BW/day, FAA received fluoroenylacetamide (FAA) at 40 μ g/day, while RFO-FAA received both RFO and FAA. At 4 weeks blood samples were taken from the tail. At 8 weeks the rats were sacrificed for collection of blood and liver tissues. Ellman's method was employed to determine the parameters of antioxidant glutathione (glutathione sulfhydryl/GSH). One-way ANOVA and Tukey post hoc test were used to compare glutathione levels between groups.

RESULTS

This study showed that liver and plasma glutathione levels were not significantly lower in the RFO-FAA group than in the FAA group. Glutathione levels were significantly lower in plasma and liver homogenates of the RFO group compared with the control group and were not significantly different from those in the FAA group.

CONCLUSION

Administration of RFO in rats does not protect against decreased glutathione but is a potential source of oxidative stress.

Key words : Red fruit oil, oxidative stress, N-(2-Fluorenyl) acetamide, glutathione, rats

Minyak buah merah tidak mampu mencegah terjadinya stres oksidatif pada tikus

ABSTRAK

LATAR BELAKANG

Minyak buah merah (RFO) banyak dikonsumsi masyarakat Indonesia khususnya Papua untuk kesehatan dan pengobatan beberapa penyakit. RFO dilaporkan mengandung antioksidan α -tokoferol dan β -karoten. Penelitian ini bertujuan menilai efek perlindungan RFO untuk mencegah penurunan kadar antioksidan glutathione dalam plasma dan jaringan hati tikus yang diinduksi N-(2-fluorenyl) acetamide (2-FAA).

METODE

Tikus jantan Wistar dengan berat 200 ± 20 g dibagi secara acak dalam 4 kelompok perlakuan eksperimental ($n=6$ tikus/kelompok) yaitu kelompok yang hanya diberi aquades (kontrol), 10 ml/g/BB/hari RFO, diinduksi 40 mg/hari fluorenylacetamide (FAA) dan kelompok yang diberi 10 ml/g/BB/hari RFO dilanjutkan dengan induksi 40 mg/g/fluorenylacetamide (RFO+FAA). Setelah perlakuan 4 minggu, darah diambil dari ekor tikus dan pada minggu ke-8 semua tikus dimatikan. Pemeriksaan kadar glutathione (glutathione sulfhydryl/GSH) menggunakan metode Ellman. One-way ANOVA dilanjutkan dengan uji post hoc Tukey digunakan untuk membandingkan kadar glutathione antara keempat kelompok perlakuan.

HASIL

Hasil pengukuran dalam plasma dan jaringan hati menunjukkan bahwa kadar GSH plasma dan jaringan hati tikus kelompok RFO+FAA menurun tidak berbeda bermakna dibandingkan FAA. Dari penelitian ini juga terungkap bahwa kadar glutathione dalam plasma dan jaringan hati kelompok RFO lebih rendah bermakna dibandingkan kontrol dan tidak berbeda bermakna dibandingkan kelompok FAA.

KESIMPULAN

Pemberian RFO pada tikus ternyata tidak memiliki kemampuan mencegah turunnya kadar glutathione dan justru berpotensi menyebabkan stres oksidatif.

Kata kunci : *Minyak buah merah, stres oksidatif, N-(2-Fluorenyl) acetamide, glutathione (GSH), tikus*

INTRODUCTION

An imbalance in the production of free radicals and antioxidant defenses in the body is termed oxidative stress, causing oxidative damage in cellular macromolecules.^(1,2) However, dietary manipulation with antioxidant-containing foods may prevent oxidative stress.⁽²⁻⁴⁾

Red fruit oil (RFO) and other traditional medications as antioxidant supplements for the prevention of various degenerative diseases are now beginning to become popular in Indonesia.⁽⁴⁻⁶⁾ RFO supplements are already

widely circulated and consumed by the community. RFO is reported to contain a number of antioxidants, such as α -tocopherol, β -carotene, and other phenolic and flavonoid compounds.^(6,7) In vitro and in vivo studies have demonstrated the antioxidant activity of RFO.^(8,9)

Glutathione (glutathione sulfhydryl/GSH) is one of the intracellular antioxidants produced by the body and acts to prevent oxidative damage due to free radicals.^(11,12) A previous study induced oxidative stress in rats by means of N-2-fluorenylacetamide (2-FAA) 40 mg/day

for 8 weeks, resulting in a significant decrease in glutathione concentrations as compared to controls.⁽¹⁰⁾ The objective of the present in vivo experimental study was to evaluate the effect of RFO supplements in preventing decreases in plasma and hepatic tissue GSH levels in rats with oxidative stress induced by N-2-fluorenylacetylamide (2-FAA).

Study design

This experimental study was conducted in the Biochemistry Laboratory of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia, from September until November 2006.

Experimental animals

The number of animals required (24) was calculated according to Frederer's formula, viz. $(n-1)(k-1) \geq 15$. The animals were two-month old male Wistar albino rats (*Rattus sp*) weighing 200 ± 20 g, obtained from the Veterinary Research Institute (*Balai Penelitian Veteriner*), Bogor, West Java. The animals were kept in the animal house of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia.

Interventions

The Wistar rats were randomized into a control group (receiving distilled water only) and three intervention groups, designated RFO, FAA, and RFO+FAA. Group RFO received RFO at 10 μ g/BW/day and group FAA received fluorenylacetylamide (FAA) at 40 μ g/day, while group RFO+FAA received both RFO (10 μ g/BW/day) and FAA (40 μ g/day). In the last group, FAA was given 2 hours after administration of RFO.

The interventions were administered daily for 8 weeks, except for RFO administration in the RFO+FAA group. In this group RFO was given one week before induction with FAA, in order to ensure the protective effect of RFO. All interventions were given by gavage. The

RFO dosage of 10 μ g/BW/day was a conversion of the usual dose for human consumption of 3 spoonfuls per day for administration to the rats.

Biochemical measurements

At the end of the fourth week of intervention, blood samples were collected from the rats by snipping off the tips of their tails, after an antiseptic swab to prevent infection, and collecting the blood into tubes containing ethylene diamine tetraacetic acid (EDTA). In the eighth week of intervention, all rats were sacrificed using ether. The anesthetized rats had their legs fully extended and were fixated by pinning the legs to a dissecting board. After antisepsis of the thorax and abdomen, the thoracic cavity was opened and the heart wetted with 3 drops of heparin solution, followed by cutting the cardiac apex with scissors and collecting the blood by means of a Pasteur pipette. Subsequently the liver was removed from the surrounding tissues by blunt dissection. The blood in the EDTA tubes was centrifuged at 3000 rpm for 10 minutes to separate the plasma, which was stored in a freezer at -20°C until required.

For measurement of GSH concentrations, one gram of rat liver was ground in NaCl 0.9% into a homogenous mass. GSH concentrations in plasma and the homogenized liver tissue were then determined by a modification of Ellman's method in the Biochemistry Laboratory of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Indonesia. The principle of GSH measurement is to react the test material with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). If the test solution contains a compound with sulfhydryl (-SH) groups, the compound will reduce the DTNB, causing the latter to be converted into 2-nitro-5-thionitrobenzoate, which in an alkaline environment absorbs light maximally at a wavelength of 412 nm, thus giving a yellow color.⁽¹⁰⁾

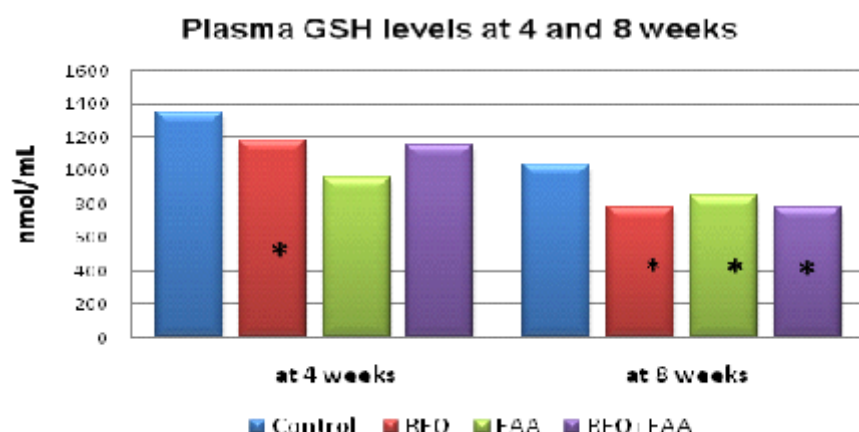


Figure 1. Plasma GSH levels at 4 and 8 weeks
 *p<0.05 compared to control group; RFO = red fruit oil; FAA = N-2-fluorenylacетamide

Data analysis

The data on glutathione concentrations were tested for normality of distribution using a standard test of normality. The normally distributed data were then tested using one-way ANOVA at a significance level of p=0.05. In the case of significant between-group differences in plasma and hepatic glutathione levels being found at 4 and 8 weeks, the analysis was to be followed by a post hoc Tukey test.

Ethical clearance

The study protocol was approved by the Research Ethics Commission, Faculty of Medicine, Atma Jaya University.

RESULTS

Induction with N-2-fluorenylacетamide in groups FAA and RFO+FAA resulted in significantly lower concentrations of hepatic glutathione as compared with controls (p=0.004). Similar results were obtained for plasma glutathione at 4 and 8 weeks (p=0.001, p=0.027) (Figure 1). The glutathione concentrations in the liver and plasma of group RFO+FAA were not significantly different from those of group FAA at each time of observation. The results even showed that the liver glutathione concentrations of group RFO at 8 weeks were significantly lower than those of and were not significantly different from those of group FAA (Figure 2).

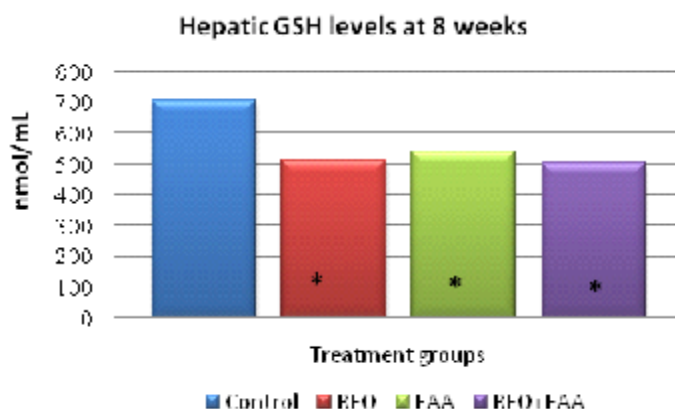


Figure 2. Hepatic GSH levels at 8 weeks
 * p<0.05 compared to control group; RFO : red fruit oil; FAA : N-2-fluorenylacетamide

The decreases in glutathione levels in the liver and plasma were similar, indicating that damage of liver tissues was reflected systemically in the plasma.

DISCUSSION

The decrease in glutathione concentrations in group FAA was caused by the glutathione neutralizing the free radical aryl nitrenium, which was formed in the bioactivation process of *N*-2-fluorenylacetamide. Glutathione conjugates with aryl nitrenium either spontaneously or by means of a reaction catalyzed by GSH-S transferase. The lower levels of glutathione in group FAA provides evidence that induction with *N*-2-fluorenylacetamide at 40 µg/animal resulted in oxidative stress.⁽¹⁰⁻¹²⁾

The results of this study show that administration of RFO in the group of rats receiving *N*-2-fluorenylacetamide was evidently not capable of preventing a decrease in glutathione concentrations, either at 4 weeks or at 8 weeks, in both plasma and liver. Moreover, this study also reveals that RFO supplementation was associated with lower glutathione levels as compared with controls and that these levels were not significantly different from those of the group that had been given FAA. Thus the present study yielded results that are at variance with the reported antioxidant effects of RFO in previous in vitro⁽⁸⁾ and in vivo studies.^(9,13)

On the other hand, the results of the present study are similar to those of an in vitro study comparing the antioxidant potency of several kinds of RFO in sheep erythrocytes that had been subjected to oxidative stress by induction with the oxidator tertbutyl hydroperoxide (TBHP).⁽¹⁴⁾ The decrease in the glutathione antioxidant levels on administration of RFO may possibly have been caused by the oxidants originally present in RFO or oxidants formed by the prolonged high temperatures employed in the extraction of the oil.⁽¹⁵⁾ The glutathione antioxidants may have been used to

neutralize these oxidants, leading inevitably to decreased glutathione levels. The decreased glutathione in group RFO may also have been caused by impaired GSH synthesis as a result of liver damage.^(16,17) Other studies have shown that RFO supplementation increases plasma SGPT concentrations.^(18,19)

To account for the oxidative stress due to RFO it may be assumed that the dosage of the RFO administered in the present study was relatively high and that the high levels of vitamins, minerals and antioxidants present in RFO may together act as prooxidants by inducing Fenton and Haber-Weiss reactions, thus increasing the production of hydroxyl radicals and decreasing the antioxidant levels in the blood.⁽²⁰⁻²²⁾ Another explanation may be that the high amounts of fatty acids and carotenoids in RFO can activate microsomal oxidases thus resulting in bioactivation of toxic substances or fatty acid degradation products caused by the prolonged heating of the red fruits for oil extraction.⁽²³⁻²⁵⁾ The present study demonstrates the potential for oxidative stress of RFO and therefore further studies on RFO are highly desirable.

CONCLUSION

Administration of commercially available red fruit oil at 10iL/gBW/day to rats induced with *N*-2-fluorenylacetamide at 40mg/animal/day for 8 weeks was unable to prevent oxidative stress, but was in contrast potentially capable of causing oxidative stress through decreased plasma glutathione levels.

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