"TEMPE" REDUCES DNA DAMAGE IN RATS IRRADIATED WITH ULTRAVIOLET RAY

Siti Maryam [1], A.A.G.Sudewa Djelantik[2],
I Nyoman Agus Bagiada[2], Nyoman Mantik Astawa[3]

[1]Postgraduate Program, Udayana University
[2]Faculty of Medicine, Udayana University
3Faculty of Veterinary Medicine, Udayana University
E-mail: titik-maryam@yahoo.co.id

ABSTRACT

'Tempe' is a popular Javanese-Indonesian tradisional food made of fermented soyabean. This study aims to examine whether 'tempe', used as a strong anti-free, has the ability to decrease DNA damage induced by ultraviolet ray irirradiation in the Wistar rats as indicated by the levels of urinary 8-hydroxy-2-deoxyguanosine.

A Total of 24 Wistar rats, 2.5 to 3 mounths and 200 gr body weight, were dividing into 4 groups of equal size i.e. three treatment and one control groups. All rats were exposed to 5-hours daily UV ray sources of UV 15 watt Philip for 60 days. Randomized post test only control group design was used in this study with independent variables of 0, 1, 2 and 3 g 'tempe' per kilogram body weight per day and independent variables of 8-hydroxy-2-deoxyguanosine urinary level as a DNA damage biomarker following an oxidative stress. Data were analyzed by using one way ANOVA followed by Tukey HSD.

The results of our study indicates that there is a decrease of DNA damage is manifested by progressive decrease of 8-hydroxy-2-deoxyguanosine level in the treatment groups as compared to the control group. Statistically significant decrease of 22.61 % and 25.62 % was shown with $p > 0.05$.

From our analysed data is could be inferred that 'tempe' has the ability to decrease DNA damage caused by ultraviolet ray irradiation. Supplementation of 2 g 'tempe' per kilogram body weight per day appeared to have the strongest effect of decreasing DNA damage in Wistar rats.

Key words: Tempe, fermented soybean, ultraviolet ray irirradiation, 8-hydroxy-2-deoxyguanosine, DNA-damage

INTRODUCTION

Oxidative stress is a condition in which there is an inbalance of body antioxidant and oxidant [1,2,3]. During stress oxidative, patholgic oxidation reaction occures, originating with the present of oxidative free radical [4].

Non radical oxidant and free radicals that have properties demaging cells can be initiated from many sources [5]. 1) intern or through biological process; 2) extern such as medicine and pollutant; 3) irirradiation(X-ray), UV ray; and 4) cells trigering inflamation.

The present of free radicals on human body, can be controlled by a defence system known as scavenger using antioxidant as a free radicals muffle. Antioxidant compromises of endogen produced by the body it self and exogen sourcing from out side, such as food.[4]

Natural antioxidant originates from phenolic and isoflavon components that can be found in all of plant, i.e. wood, root, flower, and pollen. For example, the use of *atung* fruit [6],; likopen[7]; soybean isoplavon [8,9,10,11]; 'tempe'[11], and the present of Superoxide dismutase (SOD) like on 'tempe' Tunggak.[12]

'tempe' is a traditional food known since ages in Indonesia. There are many health advantages of 'tempe', therefore, 'tempe' is a functional food that has a positive impact on some one health [13]. [14]stated that 'tempe' can be used as an antioxidant. Antioxidant content of 'tempe' are isoflavon, vitamin E, and SOD. The present isoflavon genestein (112 μg/g); daidezein (724 μg/g); and 8-hydroxy daidzein (824 μg/g). SOD and vitamin E of 'tempe' are 1.24 μmol/g) and 1125 IU/g, respectively [15].

In normal condition, free radicals in the body can be inhibited by defence system due to there is an enzymatic antioxidant, such as SOD, catalase, glutathion peroxidase which an ability to combat oxidant that enter the body. However, in a certain condition that trigger an over free radical, such as over exposure of ultraviolet, and global warming, and junk food consumption, which is leads to the defence system is unable to muffle free radical complately, so that there will be a pathologic deffect caused by free radicals.

One causing of the present of free radical in the body is as a results of irradiation such as ultraviolet irirradiationor sun ray exposure. Some negative impacts of ultraviolet irirradiation are lipid peroxidation [16]; stress oxidative[17]; eritema [18]; early aging, skin cancer and induction of skin pygmentation[19,20]. Negative impact of this irirradiation is 70% due to free radicals, i.e hidoxyl radical. Hydroxyl radical is a very reactive free radical that can cause lipid peroxidation, cross linked of protein, and DNA damage marked by increase of 8-hydroxy-2-deoxyguanosine level in serum [21].

The use of 'tempe' as an antioxidant is due to the content of bioactive compounds, such as isoflavon in the form of daidzein and genistien[22]. In addition, there is also due to the present of SOD like[23]. Genestein can be used as a chemoprevention as a results of anti estrogen property and can also acts as tirosin kinase inhibition [24]. Besides, 'tempe' is food containing [25], therefore, 'tempe' is a functional food that can be used as an alternatif for inhibiting DNA damage due to oxidative damage by free radicals or exposure of ultraviolet ray irradiation.

MATERIAL AND METHOD

A number of 24 Wistar rat age of 2.5-3 months and weight of 200 g are randomizely divided into 4 groups (0, I, II, and III) and adapted for 15 days with standar diet and drink ad libitum. Then, at 0.9.00 until 14.00 all of the rats were exposured to UV ray sources of TL UV 15 watt Philip for 60 days. Before irradiation, all af the rats were supplemented with 'tempe' in various concentration, i.e. 0 g, 1 g, 2 g and 3 g per kilogram body weight per day. After irirradiation of 60 days, the urinary of the rat was collected during 24 h for determination of 8-hidroxi-2-deoxyguanosine by employing ELISA.

RESULTS

Concentration of urinary 8-hydroxy-2-deoxyguanosine of 24 rats radiated with UV which were supplemented with *'tempe'* of 0 g, 1 g, 2 g, and 3 g per kilogram body weight per day before are listed in Table 1.

Table 1 Urinary Concentartion of 8-hydroxy-2-dioxyguanosine in Wistar Rats Urinary Radiated with UV Ray Supplemented with 'tempe'

No	P0	P1	P2	P3
1	18.00	16.74	13.90	13.00
2	18.92	16.54	12.59	12.18
3	19.16	16.34	13.00	12.19
4	17.64	17.14	12.59	12.72
5	17.14	17.22	12.72	12.45
6	18.60	16.33	13.00	12.23
∑	109.47	100.31	77.80	74.78
Mean	18.24±0.79	16.76±0.39	12.97±0.49	12.46±0.34

Remarks: P0 supplementation of 0 g 'tempe'per kilogram body weight per day
P1 supplementation of 1 g 'tempe'per kilogram body weight per day
P2 supplementation of 2 g 'tempe'per kilogram body weight per day
P3 supplementation of 3 g 'tempe'per kilogram body weight per day

One way variance analysis indicates that there is a significance different of urinary 8-hidroxi-2-deoxyguanosine concentration of Wistar rat between control and treatment group with 0 g, 1 g, 2 g, and 3 g *'tempe'* supplementation per kilogram body weight perday ($p < 0.05$).

HSD Tukey test reveals that there is a significant decrease of 8-hidroxi-2-deoxyguanosine concentration for P1, P2, and P3 compared to control group ($p < 0.05$). There is also a significant decrease different of 8-hydroxy-2-deoxyguanosine concentration between P2 and P3 ($p < 0.05$). However, no significant decrease observed between P2 and P3 as indicated by $p > 0.05$

DISCUSSION

Compound of 8-hydroxy-2-deoxyguanosine is a product reaction of hidoxyl free radical (^{0}OH) with DNA. The free radical in mamalian Wistar rat is radiated with UV. The free radical is an atom or collection of atom (molecule) with one or more unpaired electron. The unpaired electron behaves to form paired by gaining one electron from other compound to form a new radical.[3]

The present of the unpaired electron resulted in this compound to be reactive to gain pair by attacking and bond electron of molecule in their sorrounding. If the electron bonded the free radical behaves as an ionic, so the the impact is not dangeroune. However, if it originates from covalen bonding compound, it will lead to demaging cell due to sharing of bonding on their external orbital. Generally, compound with covalen bound are macromolecule, such as protein, lipids, and DNA.

Many researchs state that the guanine of the DNA is the main target of free radical species (ROS) as an oxidation of atom carbon no.8 (C8) of the base to form 8-hydroxi-2-deoxyguanosine (8-OHdG) [21] and that compound known as a biomarker for demaging or recovering oxidative DNA.

Based on this reference, in this research after statistical analyzed, it can be stated that supplementation of 'tempe' in P1, P2, and P3 treatment could decrease significantly 8-OHdG concentration compared to control group P0 ($p < 0.05$).

Decrease of 8-OHdG concentration between P0 and P1 is 8.15%; and between P0 and P2 is 28.92%; and between P0 and P3 is 31.69%.

There is a decrease of 22.61% of 8-OHdG concentration between P1 compare to P2, also for P1 and P3 is about 25.62%. For P2 in comparison to P3, there is no significant different indicated by $p > 0.05$. This is due to free radical in the body of the rat has already in saturated level, therefore, antioxidant intake would not produce high muffle. The result of this research are supported by Ogawa et al. (2006); Borchers et al., 2006; and Subas et al. (2010) that stated an antioxidant will giving maximum effect depending on concentration of the antioxidant.

In general, it can be explained that the present of 'tempe' supplementation in Wistar rat irradiated with UV ray through TL UV Philips 15 watt for 5 h everyday for 60 days affects urynary rat 8-OHdG concentration. This is as a results of 'tempe' contain of antioxidant with compromise of varies isoflavon, i.e. genestein, daidzein and 8-hidoxy daizein, SOD, and vitamin E (α-tocoferol). All of these antioxidants have poly phenol species in their structure. This poly phenol species will attach free radical caused by UV irradiation. Increasing supplementation to the rats will increase isoflavon content and also increase poly phenol, therefore it has a greater ability to accept free radical (scavenger).

SOD is a component of antioxidant found in 'tempe'. The present of SOD which is an enzimatic antioxidant functional to attach free radical caused by irradiation. In addition, the present of SOD leads to singlet oxygen changes to hydrogen peroxyde and reducing their free radical behaviour. This leads to no propagation reaction or elongation chain of free radical occures, therefore, no further free radical reaction could be stopped [27].

Vitamin E (α-tocoferol) is a non enzimatic antioxidant. The present of vitamin E in 'tempe' leads to 'tempe' has greater antioxidant behaviour and function as a more potent antioxidant as a cause of phenolic species on its structure, therefore, its ability to attach free radical will increase.

The comparison of P1 group to P2, it was obtained that there is a significant different, which mean that increase supplementation of 2 g 'tempe'per kilogram body weight can also decrease DNA damage caused by UV irradiation. This is as a results of intake of 2 g 'tempe'per kilogram body weight with compromise of 44.8 μg genestein, 289.6 μg daidsein, 329.0 μg 8-hydroxy daidzein, 0.496 mmol SOD, and 450 IU vitamin E [15] can muffle free radical caused by UV irradiation. Evidence from this research is 8-OHdG concentration for P2 obtained about 12.97 ng/mL and for P1 about 16.76 ng/mL. Increasing 'tempe' supplementation to animal will increase antioxidant presence. Therefore, ability to muffle free radical will also increase, as evidence that there is a decrease of 22.61% of 8-OHdG levels, a marker of DNA damage by hydroxyl free radical.

No significance different obtained between treatment 3 (P3) intake of 3 g *'tempe'*per kilogram body weight per day compared to treatment 2 (P2), indicated by $p = 0.378$. Intake of 3 g *'tempe'* has a consequence, that there is antioxidant content of 67.2 μg genestein, 434.4 μg daidzein, 494.4 μg 8-hydroxy daizein, 0.744 mmol SOD and 675 IU vitamin E [15]. Therefore, addition of antioxidant with compromise of 22.4 μg genestein, 144.8 μg daidzein, 164.8 μg 8-hydroxy daizein, 0.246 mmol SOD and 225 IU vitamin E did not significantly decrease DNA damage any further ($p > 0.05$). This is due to intake of 2 g 'tempe'per kilogram body weight per day has already perfom maximum free radical muffling, therefore, addition much more 'tempe' did not significantly decrease DNA damage. In other words, intake of 2 g 'tempe'per kilogram body weight per day reveals maximum concentration of antioxidant content in 'tempe', i.e. 44.8 g genestein, 289.6 μg daidsein, 329.0 μg 8-hydroxy daidzein, 0.496 mmol SOD, and 450 IU vitamin E [15]. Vitamin E (α-tocoferol) is one of vitamin behave as antioxidant and prooxidant, in which in high concentration will leads to a dangereous condition. Maximum intake dose of vitamin E per day is 450 IU [29].

CONCLUSSION

'Tempe' supplementation results in decrease of DNA damage of Wistar rat irradiated with UV ray marked by decrease of 8-OHdG levels. Decrease of DNA damage among control (P0) and P1, P2, P1nd P3 are 8.15%, 28.92%, and 31.69, respectively ($p < 0.05$). Supplementation of 2 g per kilogram body weight perday 'tempe' gives the strogest effect of decreasing DNA damage in Wistar rat irradiated by UV ray.

SUGGESTION

In correlation reduce consentration 8-hydroksi-2-deoxyguanosine urinary than reduce level malondialdehida (MDA) due to suplementation 'tempe'.

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