Kasetsart J. (Nat. Sci.) 36 : 166 - 174 (2002)

Acute Toxicity, Mutagenicity and Antimutagenicity of Ethanol *Ocimum sanctum* Leaf Extract Using Rat Bone Marrow Micronucleus Assay

Ekachai Khumphant[1] and Darunee Buripakdi Lawson[2]

ABSTRACT

Holy basil (*Ocimum sanctum*) is an herbal plant that is commonly used as an ingredient for cooking and as a medicinal plant. This plant is widely used in all Thailand regions for many food recipes. Previous studies have shown that *Ocimum sanctum* leaf extract (OE) possesses chemopreventive property against carcinogenesis and induction of the hepatic biotransformation enzyme profiles that are involved in detoxification of chemical mutagens. This study attempts to investigate the effect of an oral administration of OE on induction of toxicity, mutagenicity, and anti-mutagenicity using rat bone marrow micronucleus test. Cyclophosphamide was used to elucidate the possible mechanism of anti-mutagenicity property of this extract. OE was given daily via oral administration to Sprague-Dawley rats to determine; (1) acute toxicity, (2) mutagenicity, and (3) anti-mutagenicity against cyclophosphamide. The results shown that OE was not toxic up to the dose of 15 g/kg bw in 5-week-old rats. The mutagenicity, represented as micronucleus induction, was assessed by bone marrow using i.p. 80 mg/kg bw of cyclophosphamide 30 hours prior to bone marrow collection. Administration of OE at a dose 5 g/kg bw did not cause mutagenicity in the rats. On the other hand OE possesses the anti-mutagenicity activity resulting on the significant inhibition of micronucleus formation against cyclophosphamide after 7 and 21-day repeat oral administration of 5 g/kg bw of OE. The anti-mutagenicity of OE suggested an enhancement of detoxification enzymes against cyclophosphamide as shown in a reduction of micronucleus formation. It is possible that some compounds in OE may play an important role in such enhancement of detoxification mechanism. This study, therefore, confirmed health benefits of holy basil (*Ocimum sanctum*), especially grown in Thailand as an ingredient of food and as a medicinal plant to reduce mutagenicity.

Key words: *Ocimum sanctum*, anti-mutagenicity, mutagenicity, micronucleus, acute toxicity

INTRODUCTION

Constituents of the human diet that inhibit mutagenesis and carcinogenesis are of particular importance because they may be useful in the prevention of human cancer. *Ocimum sanctum* L. (Labiatae) popularly known as "Kaprao" in Thailand, "Tulsi" in Hindi and "Holy basil" in England has been extensively used as an ingredient for cooking and as a medicinal plant. This plant was widely used in all Thailand regions for many food recipes and claimed to be valuable against a wide variety of diseases. Preliminary evaluation of this plant was first reported by Aruna and

1 Food and Nutritional Toxicology Programme, Institute of Nutrition, Mahidol University, Nakhon Pathom 73170, Thailand.

2 Division of Research and Academic Support, National Laboratory Animal Center, Mahidol University, Nakhon Pathom 73170, Thailand.

Sivaramakrishnan (1992) demonstrated the effect of *Ocimum sanctum* leaf extract (OE) against chromosomal aberrations, neoplasia of stomach and hepatomas. The plant is also documented to have chemopreventive property against skin papillomagenesis (Prashar and Kumar, 1995). The mechanism of influence of *Ocimum sanctum* on the incidence of papillomas and carcinomas are yet to be known and the possible mechanism may be due to enhancement of the detoxifying enzyme system in animals (Karthikeyan *et al*., 1999).

Previous study has reported the modulatory influence of alcoholic extract of the leaves of *Ocimum sanctum* on the activities of cytochrome P450, cytochrome b5 and aryl hydrocarbon hydroxylase in liver. It is also reported the extract enhanced glutathione – S – transferase (GST) and a reduced glutathione level in the liver, lung and stomach of mice. All of these enzymes and cofactors play an important role in the detoxification of carcinogens and mutagens (Banerjee *et al*., 1996).

Though leaf and ethanol extracts have been reported for its chemopreventive activity, however the effect of ethanol leaf extract of *Ocimum sanctum* have not been studied so far in mutagenicity and antimutagenicity. Thus, the present study attempts to investigate the effect of an oral administration of *Ocimum sanctum* leaf extract on induction of toxicity, mutagenicity, and antimutagenicity using rat bone marrow micronucleus test.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats weighing 120±10 g were obtained from breeding colony of National Laboratory Animal Center which kept in Strict Hygienic Conventional system. Each rat was housed in stainless steel cage with dimensions of 7.5-in width, 11.5-in length and 5.0-in height, containing 2-cm wood shavings. Rats were maintained in standard conditions including relative humidity of 60±15%, 12 hour light / 12 hour dark cycle, adequate ventilation and ambient temperature of 25±2°C. Animals received commercial laboratory animal feed pellets (Chalernpokkapun Co., Ltd. No. 082), and acidified filtered water pH in the range of 2.3-2.5 *ad libitum*.

Chemicals

All chemicals used throughout this study were analytical grade. Giemsa stain and cyclophosphamide (CP) were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A. through Theera Trading Co., Ltd. The sterile normal saline solution was purchased from Thai Otsuka Pharmaceutical Co., Ltd.

Preparation of *Ocimum sanctum* leaf extract (OE) from fresh basil leaves

Insecticide free *Ocimum sanctum* plants were grown for 60 days. Botanist of Sirerukkachart herb garden of pharmacology division Mahidol University confirmed the plant classification. The fresh leaves (40kg) were cut and washed with tap water and shed dried at room temperature (28°C) overnight. The air-dried leaves were kept in 60°C oven for 5 h when constant dry weight was achieved. The dried leaves were ground into powder form using the grinding machine. Total amount of 5.721kg of the basil leaf powder was soaked in 4 l of 70% ethanol for 24 h at room temperature. The extract was filtered through filter paper no.3. After filtration the solid part was soaked again and filtered 24 h later. The amount of 13.5 l extract from three repetition of extraction using 70% ethanol as previously mentioned were pooled and evaporated with the evaporator (EYELA model NE) at 60°C until the extract was turned into dark brown of paste. The paste was dried in desicator for 2 weeks. The yield was 978.15 g.

Preparation of OE for oral gavage

Before used, the extract was dissolved in 0.9% of sterile normal saline in the amount of 500 mg/ml for dose 2500 mg/kg bw and kept in cold

room at temperature 8°C. This method was carried out according to Chattopadhyay (1999). The dissolved extract would be gavage to Sprague-Dawley rats with ball tip needle no.16 connected to five-ml syringe.

Acute toxicity test

Male and female Sprague-Dawley rats weighing 120±10 g were divided into 4 groups (two control and two treatment groups). Each group consisted of 10 animals. On day 3, 15 g/kg bw of OE dissolved in NSS was given orally to the animals. All rats were checked for theirs general behavior and the occurrence of abnormal sign were recorded after 12, 24, 36, 48, 72, and 96 hours after 15 g/kg bw of OE administration. Throughout the experiment, all animals were recorded for the body weight and food consumption. Finally, at the end of experiment (day 17), the animals were killed and autopsied to determine the toxicity effect. The toxicity of OE would be shown in number of animals died, body weight, food consumption, abnormal behavior and organs observation.

Micronucleus assay

The preparation and staining of bone marrow cells were carried out according to Schmid (1975). The experimental animals were sacrificed 30 h post-treatment, and bone marrow cells from a femur was flushed with 0.5 ml phosphate buffer saline. The bone marrow extract from each rat was centrifuged at 1000 rpm for 5 min, resuspended, and spread on to 3 coded slides, which were then stained with May-Grunwald and Giemsa as described by Schmid (1975). For each experimental point, eight rats were used and 1,000 polychromatic erythrocytes (PCEs) were score per animal from a single slide to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs). The ratio of PCE: NCE was also determined by counting the number of PCEs among 300 erythrocytes. The normal PCE: NCE ratio of bone marrow is approximately 0.4-1.0. If this ratio is ≤ 0.1, it indicates that the test substance has cytotoxic effect. For control of bias, all slides were coded prior scoring and scored blind (Heddle *et al.*, 1983).

Mutagenicity of OE using rat bone marrow micronucleus assay

Thirty-two female of Sprague-Dawley rats at age 5-wk. old weighing 120±10 g were divided into 4 groups (8 rats per group). OE was dissolved in 0.9% normal saline (NSS) to a concentration of 5 g/kg bw. Treated animals were given orally with OE at a dose 5 g/kg bw for 7 days. Negative control was treated with solvent only (NSS). For positive control, rats were intraperitoneally (i.p.) injected with 80 mg/kg bw of cyclophosphamide (dissolve in 0.9 % NSS). Untreated animal was also used to compare with the treated animals on the induction of micronucleus. All animals were killed at 30 hours post-treatment for bone marrow collection. Contents of bone marrow from a femur were smeared on the slides and then stained with May-Grunwald and Giemsa staining as previously mentioned. The frequency of MNPCEs in 1,000 PCEs per animal and the ratio of PCE: NCE in 300 erythrocytes were counted under light microscope.

Antimutagenicity of OE using rat micronucleus assay

Forty- eight Sprague-Dawley rats were divided into 3 groups. Group I was 7 or 21-day given orally with 5g/kg bw of OE and followed by intraperitoneal ingection with 80 mg/kg bw of cyclophosphamide (dissolve in 0.9 % NSS), 30-h prior to bone marrow harvesting. Group II was orally administrated of 0.5 ml/kg bw normal saline and Group III was intraperitoneally injected with 80 mg/kg bw of cyclophosphamide, which is the positive control. The micronucleus assay was carried out according to the method as previously mentioned.

Statistical evaluation

The differences of MNPCEs and PCE: NCE ratio between the control and treatment group was compared by Mann-Whitney *u*-test. For acute toxicity test, the body weight and food consumption among control and treatment were compared according to Student *t*-test. Statistical significant of difference between groups were taken at values of less than 0.01 ($p<0.01$).

RESULTS AND DISCUSSION

Acute toxicity of ethanol *Ocimum sanctum* leaf extract

This study the dose at a concentration of 15 g/kg bw of OE was given orally to the male and female Sprague-Dawley rats. The result demonstrated, there is no toxicity effect of the dose of 15 g/kg bw of OE. Observations were determined by survival of animals, body weight change, general behavior and autopsy investigated reports. The number of dead animals was not shown in this study and no significant differences in body weight were observed in OE-treated rats in both sexes compared to the control groups (Figure 1). Furthermore, the food consumption between OE-treated rats and control was not significantly different in this study (Figure 2). The general behavior observation showed no specific sign of toxicity in the animal similar to result derived from an autopsy technique that demonstrated no abnormal sign occurring in observation organs.

These results suggested that 15 g/kg bw of OE was not toxic to both sexes of Sprague-Dawley rats, supporting no toxicity of this well known flavoring plant exposure to human in food recipe or medical uses. The previous study of toxicity assessment reported that 70% alcoholic extract of 4500 mg/kg bw by oral administration is the LD50 of mice and the dose of 3.24 g/kg bw intraperitoneal causing in 50% animal death. (Bhargava and Singh, 1981). However, there is no toxicity effect of 50% alcohol extract of *Ocimum sanctum* given intraperitoneally to mice at a dose of 1g/kg bw (Dhar *et al.*, 1968) or 10 g/kg bw by subcutaneous injection (Mokkhasmit *et al.*, 1971). The dose of 15 g/kg bw of OE used in this study is higher than LD50 determined in mice (4850 mg/kg bw of OE given by oral administration) and also higher than the LD50 of mice fed 6200 mg/kg bw aqueous leaf extract of *Ocimum sanctum.*

However, the results suggested that the oral administration of 15 g/kg bw of OE did not produce any toxicity evaluated by this study using male and

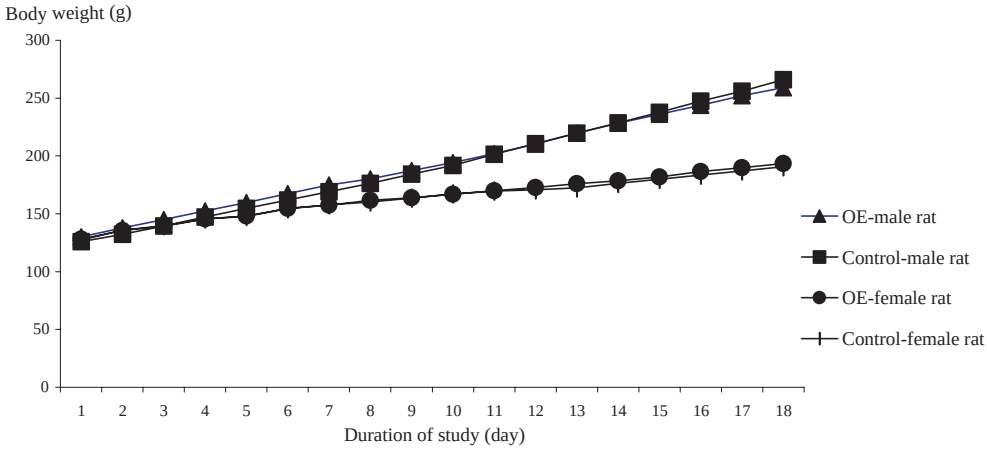

Figure 1 Body weight of male and female Sprague- Dawley rat after receiving one time of 15 g/kg bw of OE compared with control groups.There is not significantly different among OE-treated group and control in both sexes of rat.

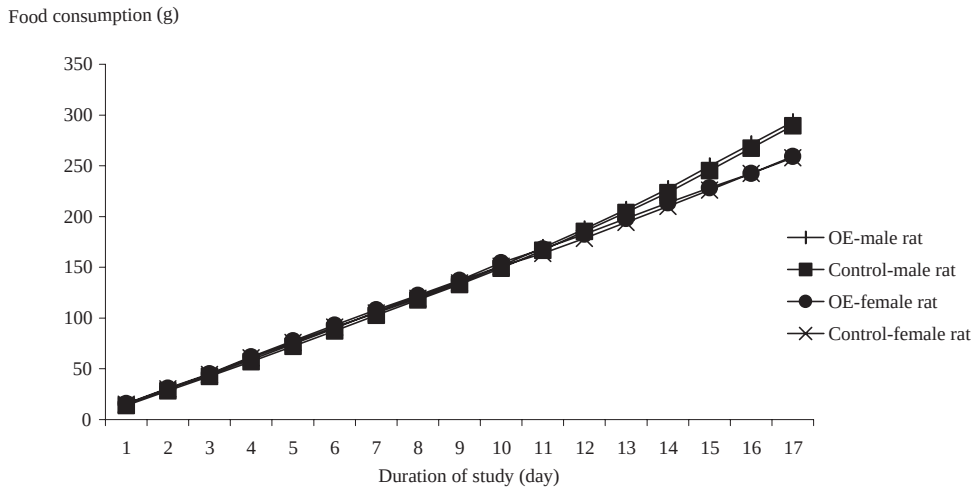

Figure 2 Food consumption of male and female Sprague- Dawley rat after receiving one timeof 15 g/kg bw of OE compared with control groups.There is not significantly different among OE-treated group and control in both sexes of rat

female Sprague-Dawley rats. These findings therefore, further research of the ethanol leaf extract of this plant on the acute toxicity or LD50 in rat was suggested in order to evaluate the toxicity applying to human exposure.

Mutagenicity of *Ocimum sanctum* leaf extract using rat bone marrow micronucleus assay

In this study, OE was tested for its potential to induced micronuclei formation in rat bone marrow cells. It showed that OE at a concentration of 5g/kg bw did not significantly increase the frequency of micronuclei formation in rat bone marrow PCEs after 7 days of oral administration to Sprauge-Dawley rat (Table 1). The ethanol extract of this plant was also reported to protect mouse bone marrow chromosome against radiation clastogenicity and did not cause mutagenicity or toxicity to the bone marrow cells (Devi *et al.*, 1998). Given with the previously supporting data of the present study, the *Ocimum sanctum* leaf extract, therefore, did not exert the mutagenic property.

Antimutagenicity of *Ocimum sanctum* leaf extract using rat bone marrow micronucleus assay

The results of the present study showed that OE at a dose of 5g/kg bw significantly decreased MNPCEs induced by cyclophosphamide compared with the control group either 7 or 21-day oral administration (Table 2, 3). It was suggested that the ethanol leaf extract of OE at a dose of 5 g/kg bw possesses the antimutagenic activity against cyclophosphamide in rat bone marrow cells.

The aqueous and alcoholic extracts from the leaves of this plant have been investigated extensively for various pharmacological activities including their activity against cancer (Prashar *et al.*, 1998). In this study, it was cleary found that an ethanol leaf extract of *Ocimum sanctum* inhibited the micronucleus formation in rat bone marrow cells. The mechanism of this property is still unknown. But several previous studies indicated the interesting possibility of the potential of this plant involving in carcinogenesis (Prashar and Kumar, 1995).

The data supports the several naturally occurring dietary or non-dietary constituents, as well as parts of several species of edible plant

Table 1 Frequencies of micronucleated polychromatic erythrocytes (MNPCEs) in rat bone marrow after 7-day oral administration of 5 g/kg bw of *Ocimum sanctum* leaf extract.

Treatment	MN PCEs/1,000 PCEs[1]	PCE: NCE ratio[2]
OE	1.88 ± 0.16*	0.53 ± 0.02*
Normal saline	1.81 ± 0.27*	0.51 ± 0.02*
Cyclophosphamide (i.p.)	39.13 ± 2.01	0.38 ± 0.09
Untreated	1.25 ± 0.28*	0.50 ± 0.02*

OE = *Ocimum sanctum* leaf extract 5 g/kg bw, Normal saline 0.5 ml/kg bw, Cyclophosphamide 80 mg/kg bw, i.p.= intraperitoneal, MNPCEs = Micronucleated Polychromatic Erythrocytes PCE = Polychromatic Erythrocyte, NCE = Normochromatic Erythrocyte
[1]Mean ± SD, n = 8, 1000 PCEs scored per animal; [2]Mean ± SD, n = 8, 300 erythrocytes (PCE/NCE) scored per animal.
* Significant difference from cyclophosphamide at p< 0.01 by Mann-Whitney *u*- test

Table 2 Inhibitory effect of 7-day oral administration of *Ocimum sanctum* leaf extract on the induction of micronucleated polychromatic erythrocytes (MNPCEs) by cyclophosphamide.

Treatment	MN PCEs/1,000 PCEs[1]	PCE: NCE ratio[2]
Normal saline	1.18 ± 0.36	0.50 ± 0.02*
Cyclophosphamide (i.p.)	39.31 ± 2.10	0.36 ± 0.09
OE interact with CP (i.p.)	25.38 ± 0.94*	0.41 ± 0.02

OE = *Ocimum sanctum* leaf extract 5g/kg bw, Normal saline 0.5 ml/kg bw, CP = Cyclophosphamide 80 mg/kg bw, i.p. = Intraperitoneal, MNPCEs = Micronucleated Polychromatic Erythrocytes PCE = Polychromatic Erythrocyte, NCE = Normochromatic Erythrocyte
[1]Mean ± SD, n = 8, 1000 PCEs scored per animal; [2]Mean ± SD, n = 8, 300 erythrocytes (PCE/NCE) scored per animal.
* Significant difference from cyclophosphamide at p< 0.01 by Mann-Whitney *u*-test

Table 3 Inhibitory effect of 21-day oral administration of *Ocimum sanctum* leaf extract on the induction of micronucleated polychromatic erythrocytes (MNPCEs) by cyclophosphamide.

Treatment	MN PCEs/1,000 PCEs[1]	PCE: NCE ratio[2]
Normal saline	1.88 ± 0.30	0.48 ± 0.10*
Cyclophosphamide (i.p.)	39.13 ± 1.89	0.37 ± 0.09
OE interact with CP (i.p.)	20.25 ± 1.80 *	0.41 ± 0.02

OE = *Ocimum sanctum* leaf extract 5 g/kg bw, Normal saline 0.5 ml/kg bw, CP = Cyclophosphamide 80 mg/kg bw, i.p. = Intraperitoneal, MNPCEs = Micronucleated Polychromatic Erythrocytes PCE = Polychromatic Erythrocyte, NCE = Normochromatic Erythrocyte
[1]Mean ± SD, n = 8, 1000 PCEs scored per animal; [2]Mean ± SD, n = 8, 300 erythrocytes (PCE/NCE) scored per animal.
* Significant difference from cyclophosphamide at p< 0.01 by Mann-Whitney *u*- test

having pharmacological activity, that may influence the hepatic biotransformation enzyme profiles that are involved in activation and detoxification of xenobiotic compounds, including chemical carcinogen (Ganasoundari *et al.*, 1997). These supporting data are evaluated the inhibitory effect of OE against micronucleus formation in this study, may involved in the hepatic biotransformation enzyme, which may play a role in detoxification of cyclophosphamide induced mutagenesis. Cyclophosphamide is an alkylating agent possessing the clastogenic effect which produced the formation of micronuclei in bone marrow cells (Countryman and Heddle, 1976). This clastogen requires activation by hepatic microsomal P450 mixed-function oxidase before being metabolized to its respective cytotoxic species, 4- hydroxycyclophosphamide, aldophosphamide, phosphoramide mustard and acroline. These metabolites of cyclophosphamide alkylate DNA, forming DNA-DNA cross linking that result in inhibit of DNA synthesis (Fleming 1997). Because genetic damage that results in chromosome break or structurally abnormal chromosome leads to micronucleus formation (USFDA, 2000), the incidence of micronuclei serves as an index of this type of damage caused from clastogenic property of cyclophosphamide. This alkylating agent is electrophilic species prone to react readily with the tripeptide glutathione through glutathione-S-tranferase. Therefore, glutathione appears to be involved directly in the detoxification of cyclophosphamide and its metabolites. Both elevate glutathione levels and increase activity of the enzyme glutathione-S-tranferase has been associated with the resistance of cells to cyclophosphamide (Colvin *et al.*, 1993). The one mechanism of this detoxification is the inactivation of the alkylating agent by conjugation with glutathione and catalyzed by glutathione-S-tranferase (Colvin *et al.*, 1993). It has been well documented that *Ocimum sanctum* leaf extract produces a substantial elevation in the hepatic microsomal cytrochrome p450, cytochrome b5 and aryl hydrocarbon hydroxylase activities in a dose-dependent manner (Banerjee *et al.*, 1996). It could effectively elevate the glutathion -S-transferase activity and the reduced glutathion levels in the liver as well as in extra-hepatic organs of mice (Barnergee *et al.*, 1996), that was consistent with Aruna and Sivaramakrishnan (1992) reported that OE increased glutathion and GST enzymes. Hence, from these accumulating evidences, it is possible that *Ocimum sanctum* leaf extract may be accelerating detoxification of cyclophosphamide in the hepatic and extra-hapatic tissues of rat by increasing the microsomal cytrochrome p450 and glutathion -S-transferase activities.

The OE was summed in this study of induction of carcinogen- metabolizing enzymes, but which component(s) of the OE enhances the enzyme activity is not clear. A principle constituent is eugenol (Prashar *et al.*, 1998) which is known to induce UDP-glucoronyl transferase and GST (Yokota *et al.*, 1988). The other compounds that found in the leaf extract may play and additional and/or synergistic chemoprotective role by inducing detoxification enzymes include sterols, triterpines, alkaloids, glycosides, saponins, tannins, various sequiterpene in essential oil of the leaves, and hydroxy chavicol (Prashar *et al.*, 1998). These compounds may influence in the reduction of the micronucleus formation induced by CP detected by rat bone marrow cells.

Thus the present finding, suggested that the 5g/kg bw ethanol leaf extract of *Ocimum sanctum* possesses the antimutagenic property determined by the significant decreasing in MNPCEs induced by cyclophosphamide in Sprague-Dawley rats. This study therefore, confirmed health benefits of holy basil (*Ocimum sanctum*), especially grown in Thailand as an ingredient of many food recipes and as a medicinal plant to reduce mutagenicity.

CONCLUSION

The conclusion of the studies could be drawn as follows:

1. For acute toxicity test, the results shown that OE was not toxic up to the dose of 15 g/kg bw in 5-week-old rats as determined by general behavior, the number of dead animal, and organ autopsy observation. The growth rates of bodyweight and food consumption in rats also demonstrated no toxicity effect of this plant at a dose of 15 g/kg bw given by oral administration.

2. The administration of OE at a dose of 5 g/kg bw did not cause mutagenicity in the rats. On the other hand, OE possesses the anti-mutagenicity activity resulting on the significant inhibition of micronucleus formation against cyclophosphamide after 7 and 21-day repeat oral administration of 5 g/kg bw of OE. The anti-mutagenicity of OE suggested an enhancement of detoxification enzymes against cyclophosphamide as shown in a reduction of micronucleus formation. It is possible that some compounds in OE may play an important role in such enhancement of detoxification mechanism. This finding, therefore, confirmed health benefits of holy basil (*Ocimum sanctum*) as and ingredient of food and as a medicinal plant to reduce mutagenicity.

ACKNOWLEDGEMENTS

This study was supported by NLAC-MU's fund for operation of the Research for Development Unit, and partially funded through the Mahidol Research Funds (national budget year 2001).

LITERATURE CITED

Aruna, K. and V.M. Sivaramakrishnan. 1992. Anticarcinogenic effect of some Indian plant products. Food Chem Toxicol. 30 : 953-956.

Banerjee, S., R. Prashar, A. Kumar, and A.R. Rao. 1996. Modulatory influence of alcoholic extract of *Ocimum sanctum* leaves on carcinogen-metabolizing enzyme activities and reduced glutathione level in mouse. Nutr Cancer. 25: 205-217.

Bhargava, K.P and N. Singh.1981. Anti-stress activity of *Ocimum sanctum* Linn. Indain J Med Res. 73 : 443-451.

Chattopadhyay, R.R. 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol. 67 : 367-372.

Colvin, O.M., H.S. Friedman, M.P. Gamsik, C. Fenselau, and J. Hilton. 1993. Role of glutathione in cellular resistance to alkylating agents. Adv Enzyme Regul. 33 : 19-26.

Countryman, P.I. and J.A. Heddle. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mut Res. 41 : 321-332.

Devi, P.U., K.S. Brisht, and M. Vinitha. 1998. A comparative study of radioprotection by Ocimum flavonoids and synthetic aminothiol protectors in the mouse. Brit J Radiol. 71 : 782-784.

Dhar, M.L., M.M. Dhar, B.N. Dhawan, B.N. Mehrota, and C. Ray. 1968. Screening of Indian plants for biological activity: Part I. Indian J. Exp. Biol. 6 : 232-247.

Fleming, R.A. 1997. An overview of cyclophosphamide and ifosfamide pharmacology. Pharmacotherapy. 17(5 Pt 2) : 146s-154s.

Ganasoundari, A., P.U Devi, and M.N. Rao. 1997. Protection against radiation-induced chromosome damage in mouse bone marrow by *Ocimum sanctum*. Mutat Res. 373 (2) : 271-276.

Heddle, J.A., M. Hite, B. Kirkhart, K. Mavournin, J.T. Macgregor, G.W. Newelland, and M.F. Salamone. 1983. The induction of micronuclei as a measure of genotoxicity. Mut Res. 123 : 61-118.

Karthikeyan, K., P. Ravichandran, and S. Govindasamy. 1999. Chemopreventive effect

of *Ocimum sanctum* on DMBA-induced hamster buccal pouch carcinogenesis. Oral Oncol. 35 : 112-119.

Mokkhasmit, M., W. Ngarmwathana, K. Sawas-dimongkol, and U. Pernphiphat. 1971. Pharmacological evaluation of Thai medical plants. J Med Assoc Thailand 54 (7) : 490-503.

Prashar, R. and A. Kumar. 1995. Chemopreventive action of *Ocimum sanctum* on 2,12 dimethylbenzanthracene DMBA induced papillomagenesis in the skin of mice. Int J Pharmacog. 33 : 181-187.

Prashar, R., A. Kumar, A.Hewer, K.J. Cole, W.Davis, and D.H. Phillips. 1998. Inhibition by an extract of *Ocimum sanctum* of DNA-binding activity of 7,12-dimethylbenz [a] anthracene in rat hepatocytes *in vitro.* Cancer Lett. 128 : 155-160.

Schmid, W. 1975. Micronucleus test. Mut Res. 31 : 9-15.

USFDA. 2000. IV.C.1.d: Mammalian erythrocyte micronucleus test. Red book 2000: Toxicological principles for the safety of food ingredients. U.S Food and Drug Administration, 15 p.

Yokota, H., H. Hashimoto, and M. Motoya. 1988. Enhancement of UDP-glucuronyl transferase, UDP-glucose dehydrogenase in rat liver by dietary administration of eugenol. Biochem Pharmacol. 37 : 799-892.

Received date : 22/04/02
Accepted date : 25/06/02