Evaluation of the Effect of Steviol on Chromosomal Damage using Micronucleus Test in Three Laboratory Animal Species

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Abstract

The chromosomal damage activity of steviol, a product of enzymatic alteration of stevioside, a natural non-caloric sweetener was reevaluated by using a bone marrow micronucleus test in both male and female hamsters, rats and mice. The micronucleus test is used widely as a rapid and efficient alternative in chromosome analysis for detecting *in vivo* cytogenetic damage. Steviol at the dose of 4 g/kg body weight for hamsters and 8 g/kg body weight for rats and mice showed no effect on the frequencies of micronucleus formation in bone marrow erythrocytes of both male and female hamsters, rats and mice. Moreover, there was also no apparent change in the PCEs:NCEs (polychromatic erythrocytes:normochromatic erythrocytes) ratio of the male animals of all three treated species at 24, 30, 48 and 72 hour intervals. However, steviol at the given dose can cause significant reduction of PCEs to NCEs ratio of the female hamsters at 72 hours and female rats and mice at 48 and 72 hours after receiving steviol orally. From these results, it could be proposed that steviol at the given dose to the treated animals produced adverse metabolites and these metabolites could reach the bone marrow, the target organ for micronucleus test. These metabolites also exhibited a slightly cytotoxic effect but not clastogenic effect to the bone marrow erythrocytes.

Key word : Steviol, Micronucleus Test, Chromosomal Damage

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Stevioside is a major sweet component of the shrub *Stevia rebaudiana* Bertoni and has been used commercially as a non-caloric sweetener in various kinds of food and food products in many countries including Japan, Korea, People's Republic of China and Brazil[1-5]. Stevioside has been subjected to various assessments for safety and no serious toxic effect has been reported[6-15]. The mutagenicity of stevioside has been studied in many test systems and no mutagenic activity of stevioside has been reported by using those systems[16-22]. However, stevioside has been demonstrated to convert to its aglycone, steviol by intestinal bacteria when given orally to rats and then steviol is nearly completely absorbed in the gastrointestinal tract[23, 24]. The mutagenic activity of steviol has also been studied in many test systems but the results are still controversial. Steviol has been determined to be nonmutagenic when tested against *Salmonella typhimurium* TA102, TA104, TA1535, TA1537, TA97, TA98 and TA100 either with or without liver S9 fractions from rats and other laboratory animal species including mice, hamsters and guinea pigs by using Ames test[19,20,22]. It also showed negative results in the bacterial reversion assay using *Escherichia coli* WP2 uvr A/pKM101 and the rec-assay using *Bacillus subtilis* either with or without the liver S9 fraction[22]. However, in contrast, Pezzuto et al[21] demonstrated that steviol was positive in the forward mutation assay using *S. typhimurium* TM677 in the presence of the liver S9 fraction from rats. Later, Matsui et al confirmed this result and also showed that steviol had positive results in *umu* test using *S. typhimurium* TA1535/pSK1002 and the gene mutation assay using CHL cell line in the presence of the liver S9 fraction from rats[22]. Besides, the liver S9 fraction from rats can activate the mutagenic activity of steviol to *S. typhimurium* TM677. Temcharoen et al demonstrated that the liver S9 fractions from mice, hamsters, guinea pigs and rabbits also activated the mutagenic activity of steviol[25]. Their results also showed that the liver S9 fraction from hamsters exhibited the highest efficiency in activating mutagenic activity of steviol while the liver S9 fraction from mice had the lowest efficiency when compared with rats, guinea pigs and rabbits[25]. For studying the effect of steviol on chromosomal damage, Suttajit et al reported that steviol at the dose up to 0.2 mg/ml showed no effect on chromosomal change in human lymphocyte culture either with or without S9 from rats[19]. But later, Matsui et al demonstrated that steviol at the dose range of 1.0-1.5 mg/ml induced chromosomal aberration in the CHL cell lines in the presence of the liver S9 fraction from rats but the result was negative in the mouse micronucleus test[22].

It has long been recognized that different species vary in their response to toxic compounds and the most susceptible species is selected in the toxicity test. It has been reported that the hamster was the most susceptible species to the toxicity of steviol than rats and mice[26]. The liver S9 fraction from hamsters also exhibited the highest efficiency while that from mice had the lowest efficiency in activating mutagenic activity of steviol [25]. Therefore, in this study, the chromosomal damage activity of steviol was reevaluated in mice and compared with hamsters and rats by using the micronucleus test. In addition, the dose of steviol in this study was higher than in the previous report.

MATERIAL AND METHOD

Adult male and female Syrian golden hamsters (80-100 g) were supplied by the Animal Production Center, Faculty of Science. Adult male and female Wistar rats (100-120 g) as well as Swiss albino mice (30-35 g) were obtained from the National Animal Center, Mahidol University, Salaya, Nakhon Pathom, Thailand. They were housed in a controlled condition at room temperature of 25 $\pm$ 2°C, relative humidity of 60 per cent, with an automatic light cycle period of 12 hours light/12 hours dark (6 A.M. to 6 P.M.). The animals were kept in stainless steel cages with a wire mesh cover and fed with Purina Lab Chow diet (Gold Coin Ltd., Singapore) and water *ad libitum*. Prior to each experiment, the animals were fasted overnight but allowed free access to water. Steviol (approximately 90% purity) was obtained by oxidation of stevioside as described by Ogawa et al[27].

Groups of 20 male and female animals of each species were gavaged with steviol (which was dissolved in corn oil) at the dose of 4 g/kg body weight for both male and female hamsters and 8 g/kg body weight for both male and female rats and mice. Cyclophosphamide (80 mg/kg body weight intra peritoneally) was used as positive control and corn oil (orally) for the negative control. All animals were killed by cervical dislocation at 24, 30, 48 and 72 hours after treatment (5 animals of

each sex from each species for each time interval). Femoral marrow cells were flushed out with fetal bovine serum and smeared on clean glass slides. Cells were fixed with methanol for 5 minutes and air dried then double stained with May-Grunwald and Giemsa[28]. The slides were coded and analyzed without any knowledge of the treatment. The frequencies of micronucleus polychromatic erythrocytes (MNPCEs) in 2000 polychromatic erythrocytes (PCEs) per animal and the ratio of PCEs to normochromatic erythrocytes (NCEs) in 400 erythrocytes were scored under light microscope.

Student's *t* test was used to calculate the statistical significance of the frequencies of micronuclei in the control and treated groups.

RESULTS

The frequencies of micronucleus polychromatic erythrocytes (MNPCEs) and the ratio of PCEs to NCEs of both male and female hamsters, rats and mice after 24, 30, 48 and 72 hours exposure to steviol at the dose of 4 g/kg body weight for hamsters and 8 g/kg body weight for rats and mice are summarized in Tables 1-3, respectively. The results showed that steviol at the dose of 4 g/kg body weight and 8 g/kg body weight did not induce a significant increase in the frequencies of MNPCEs of the treated male and female hamsters, rats and mice, respectively (Tables 1-3). The results also showed no apparent change in the ratio of PCEs to NCEs of the treated male but not female of all 3 species in each time interval (Tables 1-3). The ratio of PCEs to NCEs was significantly reduced in female hamsters at 72 hours post treatment (Table 1) and the female rats (Table 2) and mice (Table 3) at 48 hours and 72 hours post treatment. Cyclophosphamide (80 mg/kg body weight intra peritoneally), a positive control, induced significant increased in frequencies of MNPCEs in both male and female from all treated animal species. These results suggested that steviol at the given dose did not induce micronucleus in bone marrow erythrocytes of both male and female hamsters, rats and mice. However, it showed some cytotoxic effect to the female but not male of all treated animal species.

Table 1. Micronucleus test in hamster bone marrow after oral administration of steviol.

Compound	Dose (g/kg BW)	Interval (hours)	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
			male	female	male	female
Steviol	0	0	1.88 ± 0.43	1.75 ± 0.63	0.65 ± 0.04	0.73 ± 0.06
	4	24	2.00 ± 0.45	2.70 ± 0.52	0.70 ± 0.04	0.71 ± 0.04
		30	1.67 ± 0.35	2.00 ± 0.60	0.66 ± 0.03	0.67 ± 0.02
		48	2.80 ± 0.25	2.80 ± 0.64	0.64 ± 0.03	0.64 ± 0.02
		72	2.25 ± 0.75	2.50 ± 0.50	0.56 ± 0.01	0.54 ± 0.02*
Cyclophosphamide (i.p.)	0.08	30	32.6 ± 4.25**	34.0 ± 4.30**	0.65 ± 0.02	0.56 ± 0.03*

[a]Means ± SEM, n = 5-10, 2000 PCEs scored per animal, [b]Means ± SEM, n = 5-10, 400 erythrocytes (PCE + NCE) scored per animal. Significant difference from control : $p < 0.05^*$, $p < 0.01^{**}$

Table 2. Micronucleus test in rat bone marrow after oral administration of steviol.

Compound	Dose (g/kg BW)	Interval (hours)	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
			male	female	male	female
Steviol	0	0	1.00 ± 0.15	1.50 ± 0.54	0.66 ± 0.03	0.80 ± 0.04
	8	24	1.75 ± 0.55	0.80 ± 0.38	0.72 ± 0.07	0.78 ± 0.03
		30	1.00 ± 0.31	1.20 ± 0.80	0.73 ± 0.06	0.79 ± 0.05
		48	0.80 ± 0.58	2.00 ± 0.71	0.62 ± 0.05	0.54 ± 0.04*
		72	0.80 ± 0.58	1.80 ± 0.49	0.62 ± 0.04	0.65 ± 0.04*
Cyclophosphamide (i.p.)	0.08	30	17.4 ± 2.04**	16.0 ± 2.43**	0.36 ± 0.04**	0.41 ± 0.03**

[a]Means ± SEM, n = 5-10, 2000 PCEs scored per animal, [b]Means ± SEM, n = 5-10, 400 erythrocytes (PCE + NCE) scored per animal. Significant difference from control : $p < 0.05^*$, $p < 0.01^{**}$

Table 3. Micronucleus test in mouse bone marrow after oral administration of steviol.

Compound	Dose (g/kg BW)	Interval (hours)	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
			male	female	male	female
Steviol	0	0	0.65 ± 0.26	0.81 ± 0.24	0.62 ± 0.05	0.67 ± 0.05
	8	24	1.40 ± 0.49	0.80 ± 0.49	0.53 ± 0.03	0.75 ± 0.08
		30	1.00 ± 0.55	0.60 ± 0.59	0.56 ± 0.03	0.65 ± 0.06
		48	0.20 ± 0.20	0.40 ± 0.25	0.59 ± 0.04	0.49 ± 0.04*
		72	0.00 ± 0.00	0.00 ± 0.00	0.57 ± 0.03	0.49 ± 0.03*
Cyclophosphamide (i.p.)	0.08	30	31.4 ± 7.07**	28.2 ± 5.88**	0.50 ± 0.03**	0.39 ± 0.03**

[a]Means ± SEM, n = 5-10, 2000 PCEs scored per animal, [b]Means ± SEM, n = 5-10, 400 erythrocytes (PCE + NCE) scored per animal. Significant difference from control : $p < 0.05^*$, $p < 0.01^{**}$

DISCUSSION

The genotoxicity of steviol has been examined in many test systems but mostly *in vitro* system. Steviol was not mutagenic toward *S. typhimurium* TA1537, TA1538, TA97, TA102, TA104, TA98 and TA100 either with or without the liver S9 fractions from rats, mice, hamsters and guinea pigs by using Ames test[19,20,22]. It also showed negative results in *Escherichia coli* WP2 uvrA/pKM 101 by using reverse mutation assay and in *Bacillus subtilis* H17 Rec^+ and M45 Rec^- either with or without the liver S9 fraction from rats by using spore and streak rec-assay[22]. However, the result was contradictory when performed in forward mutation assay in both bacteria and mammalian cell line. Steviol showed dose-related positive response in the forward mutation assay using *S. typhimurium* TM677 in the presence of the liver S9 fractions from rats, mice, hamsters, guinea pigs and rabbits and gene mutation assay using CHL in the presence of the liver S9 fraction from rats[21, 22,25]. Steviol was weakly positive in the *umu* test using *S. typhimurium* TA1535/pSK1002 either with or without the liver S9 fraction from rats[22]. Recently, Matsui et al reported that steviol was clastogenic to cultured CHL in the presence of the liver S9 fraction from rats by using *in vitro* chromosomal aberration test, however, it did not induce micronucleus in bone marrow erythrocytes of mice after receiving steviol at the dose as high as 500 mg/kg body weight intraperitoneally and there was also no apparent change in the PCEs to NCEs ratio [22]. From these results, it might be proposed that steviol was mutagenic and clastogenic in bacteria and cultured mammalian cells in the presence of the liver S9 fraction but it showed negative response in the mouse micronucleus test. Matsui et al have suggested that it could be possible that steviol produced adverse metabolites *in vivo* but did not reach the bone marrow, the target organ for the micronucleus test[22].

From a previous study, we demonstrated that the Aroclor pretreated mouse liver S9 fraction had the lowest efficiency in activating the mutagenic activity of steviol by using bacterial forward mutation assay causing 8AG resistance in *S. typhimurium* TM 677 while the hamster liver S9 fraction had the highest efficiency when compared to the rat, guinea pig and rabbit[25]. Toskulkao et al found that hamster was the most susceptible species to the toxicity of steviol given orally when compared to the rat and mouse[26]. Therefore, the hamster is the most suitable species for genotoxicity study of steviol. In the present study, besides mice, hamsters and rats were also used to reevaluate the effect of steviol on chromosomal damage by using the micronucleus test. The result demonstrated that steviol (4 g/kg body weight for hamsters and 8 g/kg body weight for rats and mice) has no effect on micronucleus induction in bone marrow polychromatic erythrocytes of both male and female of all three species. This result is consistent with the previous report on matter whether the dose of steviol is higher than previous and the used animals are more susceptible to the toxicity of steviol. Our results also showed that the ratio of PCE: NCE was slightly reduced but was significantly different in female hamsters at 72 hours and female rats and mice at 48 and 72 hours intervals. However, these PCEs to NCEs ratio were still acceptable as normal ranges[29]. These results are controversial to the previous report by Matsui et al which showed no change in PCEs to NCEs ratio[22]. The changes in the ratio of PCEs to NCEs ratio re-

flected the cytotoxicity of the treatment[30]. Therefore, our results demonstrate that steviol may produce adverse metabolites that can reach the bone marrow of the treated animals if the dose of steviol is sufficiently high. These metabolites have only a slight cytotoxic effect but not clastogenic effect on the bone marrow cells. It has been suggested that the structurally related functional groups for expression of mutagenic activity of steviol were the hydroxy group at position 13 and the unsaturated bond joining the carbon atoms at position 16 and 17[21,31]. Compadre et al proposed that the chemically reactive metabolite of steviol should be 15-oxosteviol and it was a directing mutagen[32]. However, Procinska et al could not confirm the direct acting mutagenicity of 15-oxosteviol and suggested that the conclusion of Compadre et al was due to the misinterpetration of the data[32,33]. In addition, the enzyme epoxide hydrolase did not inhibit the mutagenic activity of steviol, indicating that the active mutagenic metabolite of steviol was not an epoxide[31]. So, the mutagenic reactive metabolites of steviol are still unknown.

The micronucleus test provides an indirect measure of the induction of structural and numerical chromosome aberration. It can serve as a rapid screen for clastogenic and test agents that interfere with normal mitotic cell division[28,34]. The mammalian micronucleus test is *in vivo* system which is used to evaluate the test compound for either clastogenic or mitotic poisoning compounds. Clastogen is a compound which is capable of inducing structural changes in chromosomes resulting in formation of acentric chromosomal fragment, a smaller size micronucleus. While mitotic poisoning compound causes non-disjunction of chromosome due to malfunction of the spindle apparatus resulting in formation of entire chromosome, a larger size micronucleus[35]. There are some findings strongly suggest that chromosomal aberration induced by chemicals are an essential event for the induction of micronucleus in the PCEs of bone marrow[36]. Micronucleus test has been proposed as a rapid and efficient alternative in chromosome analysis for detecting *in vivo* cytogenetic damage [34,37,38]. However, some limitation of using the micronucleus test to identify cytogenetic damage is the aberration that involves chromosomal rearrangement without the occurrence of an acentric fragment such as translocation, symmetrical exchanges or inversion will not be detected[39]. The results of Matsui et al[22] and our studies exhibited that steviol did not induce micronuclei in the bone marrow erythrocytes of mice, rats and hamsters. It was shown to be clastogenic to the cultured CHL cells after metabolic activation in the presence of liver S9 fraction from rats and the majority of the aberration was chromatid exchanges[22]. Thus, these aberrations may not be detected by the micronucleus test. So, further study is necessary to elucidate the question that the mammalian micronucleus test is not suitable to detect *in vitro* genetic change causing by steviol or steviol does not have clastogenic effect to the bone marrow cells. For this reason, the analysis of chromosomal aberration at metaphase in bone marrow of rodents particularly hamsters after exposure to the steviol will be appropriate for studying the clastogenic effect of steviol *in vivo*.

It can be concluded from the present study that steviol at the given dose can not induce micronucleus formation in the bone marrow erythrocytes of hamsters, rats and mice. However, it showed a slight cytotoxic effect on the bone marrow cells of the female but not male hamsters, rats and mice by reducing the ratio of PCEs:NCEs which meant that the adverse metabolites of steviol could reach the bone marrow, the target of micronucleus and might have some cytotoxic activities to the marrow cells. Since the steviol showed clastogenic effect in CHL using the *in vitro* test but did not show a clastogenic effect when using the mammalian micronucleus test. It is possible that the metabolites of steviol possess only cytotoxic effect but not clastogenic effect or the type of chromosomal changes found in the *in vitro* test can not be detected by the micronucleus test. Therefore, it requires other *in vivo* mutagenicity tests such as chromosomal analysis at metaphase in rodents for more precise assessment of the genotoxic risk of steviol to human beings.

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REFERENCES

1. Fujita H, Edahiro T. Safety and utilization of Stevia sweetener. Shokuhinkougyo 1979; 22: 66-72.
2. Kazuyama S. Present status of sweeteners from Stevia. Shokuhin Kagaku 1979; 21: 90-4.
3. Tsuchiya S. Sweeteners in pickled food. New Food Ind (Japan) 1979; 21: 12-5.
4. Kato I. Some views on the utilization and security of stevioside. Shokuhinkougyo 1975; 10: 44-9.
5. Sasaki K. Use of stevioside sweeteners in drinks. New Food Ind (Japan) 1983; 25: 38-43.
6. Planas GM, Kuc J. Contraceptive properties of *Stevia rebaudiana*. Science 1968; 162: 1007.
7. Akashi H, Yokoyama Y. Studies on the safety of stevioside extract. Shokuhinkougyo 1975; 10: 34-43.
8. Mori N, Sakanoue M, Takeuchi M, Shinbo K, Tanabe T. Effect of stevioside on fertility in rats. J Food Hyg Soc (Japan) 1981; 22: 409-14.
9. Medon PJ, Pezzuto JM, Hovanee - Brown JM, et al. Safety assessment of some *Stevia rebaudiana* sweet principles. Fed Proc 1982; 41: 1568.
10. Hagiwara A, Fukushima S, Kitaori M, Shibata M, Ito N. Effects of three sweeteners on rat urinary bladder carcinogenesis initiated by N-butyl-N-(4-hydroxybutyl)-nitrosamine. Gann 1984; 75: 763-8.
11. Yamada A, Ohgaki S, Noda T, Shimizu M. Chronic toxicity study of dietary Stevia extracts in F 344 rats. J Food Hyg Soc Japan 1985; 26: 169-83.
12. Aze Y, Toyoda K, Imaida K, et al. Subchronic oral toxicity study of stevioside in F344 rats. Eisei Shikenjo Hokoku 1991; 109: 48-54.
13. Lee SJ, Lee KR, Park IR, et al. A study of the safety of stevioside as a new sweetener source. Hangkuk Sikpum Kwahakhoe Khi 1979; 11: 224-31.
14. Xili L, Chengjiany B, Eryi X, et al. Chronic oral toxicity and carcinogenicity study of stevioside in rats. Fd Chem Toxicol 1992; 30: 957-65.
15. Yodyingyuad V, Bunyawong S. Effect of stevioside on growth and reproduction. Human Reprod 1991; 6: 158-65.
16. Okumura M, Fujita Y, Imamura M, Aikawa K. Studies on the safety of stevioside with rec-assay and reversion test. J Food Hyg Soc Japan 1978; 19: 486-90.
17. Tama Biochemical Co. Ltd. Safety of Stevia. 7-1 Nishishinjuku, 2-Chome, Shinjuku-Ku, Tokyo 160, Japan, 1981: 1-20.
18. Pimbua J, Glinsukon T, Rojanapo W, Buddhasukh D, Cheuychit P. Failure of stevioside to induce mutagenicity in *Salmonella typhimurium* TA98 and TA100 incubated with liver S-9 fractions from various species. Thai J Toxicol 1988; 4: 31-8.
19. Suttajit M, Vinitketkaumnuen U, Meevatee U, Buddhasukh D. Mutagenicity and human chromosomal effect of stevioside, a sweetener from *Stevia rebaudiana* Bertoni. Environ Health Perspect (suppl.) 1993; 101: 53-6.
20. Klongpanichpak S, Temcharoen P, Toskulkao C, Apibal S, Glinsukon T. Lack of mutagenicity of stevioside and steviol in *Salmonella typhimurium* TA98 and TA 100. J Med Assoc Thai (Suppl.) 1997; 80: 5121-8.
21. Pezzuto JM, Compadre CM, Swanson SM, Nanayakkara NPD, Kinghorn AD. Metabolically activated steviol, the aglycone of stevioside, is mutagenic. Proc Natl Acad Sci 1985; 82: 2478-82.
22. Matsui M, Matsui K, Kawasaki Y, et al. Evaluation of the genotoxicity of stevioside and steviol using six *in vitro* and one *in vivo* mutagenicity assays. Mutagenesis 1996; 573-9.
23. Wingard RE Jr, Brown JP, Enderlin FE, et al. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. Experientia 1980; 36: 519-20.
24. Nakayama K, Kasahara D, Yamamoto F. Absorption, distribution, metabolism and excretion of stevioside in rats. J Food Hyg Soc Japan 1986; 27: 1-8.
25. Temcharoen P, Pimbua J, Glinsukon T, Rojanapo W, Apibal S. Mutagenic activity of steviol to *Salmonella typhimurium* TM 677 : Comparison of the activity of S9 liver fractions from five laboratory animal species. Bull Health Sci Tech 1998; 1: 38-45.
26. Toskulkao C, Chaturat L, Temcharoen P, Glinsukon T. Acute toxicity of stevioside, a natural sweetener, and its metabolites, steviol in several animal species. Drug Chem Toxicol 1997; 20: 31-44.
27. Ogawa T, Nozaki M, Matsui M. Total synthesis of stevioside. Tetrahedron 1980; 36: 2641-8
28. Schmid W. The micronucleus test. Mutation Res 1975; 31: 9-15.
29. Adler ID. Cytogenetic tests in mammals. In: Venitt S, Parry JM, (eds). Mutagenicity testing, a practical approach. Oxford, Washington D.C., IRI press, 1984: 275-306.
30. Mavournin KH, Blakey DH, Cimino MC, Salamone MF, Heddle JA. The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res 1990; 239: 29-80.
31. Pezzuto JM, Nanayakkara NPD, Compadre CM. Characterization of bacterial mutagenicity mediated by 13-hydroxy-ent-kaurenoic acid (steviol) and several structural-related derivatives and evaluation of potential to induce glutathione

S-transferase in mice. Mutation Res 1986; 169: 93-103.

32. Compadre CM, Hussain RA, Nanayakkara NPD, Pezzuto JM, Kinghorn AD. Mass spectral analysis of some derivatives and *in vitro* metabolites of steviol, the aglycone of the natural sweeteners, stevioside, rebaudioside A and rubusoside. Biomed Environ Mass Spectrom 1988; 15: 211-22.
33. Procinska E, Bridges BA, Hanson R. Interpretation of results with the 8-azaguanine resistance system in *Salmonella typhimurium*: no evidence for direct acting mutagenesis by 15-oxosteviol, a possible metabolite of steviol. Mutagenesis 1991; 6: 165-7.
34. Haddle JA. A rapid *in vivo* test for chromosome damage. Mutation Res 1973; 18: 187-90.
35. Schmid W. The micronucleus test for cytogenetic analysis. In: Hollaender A (ed). Chemical Mutagens Volume 4 New York, Plenum Publishing Corporation, 1976: 31-53.
36. Hayashi M, Sofuni T, Ishidate M Jr. Kinetics of micronucleus formation in relation to chromosomal aberrations in mouse bone marrow. Mutation Res 1984; 127: 129-37.
37. Frank DW, Trzos RJ, Good PJ. A comparison of two methods for evaluating drug-induces chromosome alterations. Mutation Res 1978; 56: 311-7.
38. Matter BE, Grauwiler J. The micronucleus test as a simple model, *in vivo*, for the evaluation of drug-induced chromosome aberrations, comparative studies with 13 compounds. Mutation Res 1975; 29: 198-9.
39. Heddle JA, Hite M, Kirkhart B, et al. The induction of micronuclei as a measure of genotoxicity. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res 1983; 123: 61-118.

การศึกษาถึงผลของสารสตีวิออลในการทำให้เกิดความเสียหายกับโครโมโซม โดยวิธีดูการเกิดไมโครนิวเคลียสในสัตว์ทดลอง

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สารสตีวิออลเป็นสารซึ่งได้จากการเปลี่ยนแปลงของสารสตีวิโอไซด์โดยเอ็นซัยม์ สารสตีวิโอไซด์เป็นสารหวานที่ไม่ให้พลังงานซึ่งได้จากธรรมชาติ ในการศึกษาครั้งนี้เป็นการศึกษาฤทธิ์ของสารสตีวิออลในการทำให้โครโมโซมได้รับความเสียหายโดยวิธีดูการเกิดไมโครนิวเคลียสในเซลล์เม็ดเลือดแดงในไขกระดูกของสัตว์ทดลองได้แก่ หนูถีบจักร หนูพุกขาว และหนูแฮมสเตอร์ขนทองทั้งเพศผู้และเพศเมีย โดยสัตว์ทั้งสามสายพันธุ์นี้ได้รับสารสตีวิออลทางปากในขนาด 8 กรัม/ก.ก.น้ำหนักตัวสำหรับหนูถีบจักรและหนูพุกขาว และขนาด 4 กรัม/ก.ก.น้ำหนักตัวสำหรับหนูแฮมสเตอร์ จากนั้นฆ่าหนูที่เวลา 24, 30, 48 และ 72 ชั่วโมง หลังจากที่สัตว์ทดลองได้รับสารสตีวิออล และนำเซลล์ไขกระดูกจากกระดูกต้นขามาป้ายบนสไลด์ ย้อมสี และตรวจนับจำนวนเซลล์ polychromatic erythrocytes (PCEs) ที่มีไมโครนิวเคลียสใน PCEs จำนวน 1,000 เซลล์ นอกจากนี้ตรวจนับเซลล์เม็ดเลือดแดงในจำนวน 400 เซลล์เพื่อหาอัตราส่วนระหว่าง PCEs:Normochromatic erythrocytes (NCEs) จากผลการทดลองพบว่าสารสตีวิออลในปริมาณที่ให้กับสัตว์ทดลองทั้ง 3 สายพันธุ์ไม่มีผลทำให้ความถี่ในการเกิดไมโครนิวเคลียสในเซลล์ PCEs เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุมในทุกระยะเวลาที่ทำการศึกษาและทุกสายพันธุ์ของสัตว์ทดลองที่ใช้ศึกษาทั้งเพศผู้และเพศเมีย นอกจากนี้ยังพบว่าไม่มีการเปลี่ยนแปลงในอัตราส่วนของ PCEs:NCEs ของสัตว์ทดลองทุกสายพันธุ์เพศผู้ แต่อย่างไรก็ตามสำหรับแฮมสเตอร์เพศเมียพบว่าอัตราส่วนของของ PCEs:NCEs ลดลงที่ระยะเวลา 72 ชั่วโมง และหนูพุกขาวและหนูถีบจักรเพศเมีย อัตราส่วนของ PCEs:NCEs ลดลงที่ระยะเวลา 48 และ 72 หลังได้รับสารสตีวิออล จากผลการทดลองนี้อาจกล่าวได้ว่าสารสตีวิออลในปริมาณที่ให้กับสัตว์ทั้ง 3 สายพันธุ์จะถูกเปลี่ยนแปลงได้เป็นสารเมตาโบไลท์ออกมาและสารเมตาโบไลท์ดังกล่าวนี้สามารถแพร่กระจายเข้าไปถึงไขกระดูกได้แล้วไปมีฤทธิ์ฆ่าเซลล์ได้แต่ไม่มีฤทธิ์ไปทำให้เกิดความเสียหายกับโครโมโซมเมื่อทดสอบโดยวิธีดูการเกิดไมโครนิวเคลียส

คำสำคัญ : สตีวิออล, ไมโครนิวเคลียส, โครโมโซมเสียหาย

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