Available online at www. scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2020, 12 (8): 13-22
(http: //scholarsresearchlibrary. com/archive. html)

ISSN 0975-5071
USA CODEN: DPLEB4

Role of Propolis against Monosodium Glutamate Genotoxicity by Chromosomal Aberration, Micronucleus Test and Comet Assay in Males

Nagla Z. El-Alfy[1], Amany I. Alqosaibi[2], Mahmoud F Mahmoud[1], Asmaa A Emam[1*]

[1]*Department Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt*

[2]*Department of Biology, College of science, Imam Abdulrahman bin Faisal University, P. O. Box 1982, Dammam, 31441, Saudi Arabia*

[*]***Corresponding author:*** *Asmaa Ahmed Emam, Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Cairo, Egypt, Tel: +201018387665; E-mail: asmaemam2014@gmail.com*

ABSTRACT

Background: *Propolis is a natural honeybee's product which produced to protect its hive, so, it is anti-oxidant, anti-inflammatory and free radical scavengers. This work aimed to study the role of propolis against genotoxic effects of monosodium glutamate (MSG) on bone marrow chromosomes and DNA content of male albino mice. The mice divided into Group 1 were a control group, Group 2 treated with 1 g/kg body weight (b.wt.) of MSG, Group 3 treated with a dose of MSG in addition to 100 mg/kg b.wt. of propolis and Group 4 treated with a dose of propolis. All animals treated daily for two and four weeks and orally of selected doses.*

Results: *MSG treatment showed a time dependent significant increased ($p<0.001$)in chromosomal aberrations, these aberrations were centromeric attenuation, deletion, ring, fragment, centric fusion, beaded chromosome and polyploidy. Micronucleus test showed bone marrow cells toxicity which indicated by reduction of the ratio of PCE and showed highly significant increased ($p<0.001$) in % DNA damage which measured by % DNA tail comet, while the treatment with MSG in addition to propolis significantly decreased ($p<0.05$) these alternations in chromosomes and DNA.*

Conclusion: *The present study indicated that MSG treatment induced genotoxic effect on bone marrow chromosomes, DNA content of male albino mice and propolis treatment improved these changes. Therefore, the using of MSG should be restricted to a very narrow range border and may using of propolis in our daily life.*

Keywords: Chromosomes, Comet, DNA, Micronucleus, Mice.

INTRODUCTION

Monosodium glutamate (MSG) is a commonly used as flavor enhancing food additive and used in many packaged foods, fast foods and restaurant foods. Also, chips and noodles [1]. MSG is a toxic on central nervous system, adipose tissue, hepatic tissue and reproductive organs in numerous animal studies MSG is a salt of glutamic acid utilized by receptors of glutamate in mammals and these receptors are found in the heart, nerve terminals and cardiac ganglia. Also, present in the kidney, liver, lung, spleen and testis [2,3].

In the animal studies which used the same doses and extra doses intaking by human of MSG found that the treatment of MSG induced increases in insulin, fatty acids and triglycerides in blood. Also, increases the expression of some genes related to adipocytes differentiation, transaminase levels and bile synthesis in liver [4]. The MSG treatment affects on DNA content, this examined by study which revealed with DNA damage by using comet examination and found that the treatment with MSG induced significant increased (p<0.05) in the tail moment of comet compared with control group [5].

Propolis is a honeybee product (*Apis mellifera*) and its structure is very complex and differ with location and plant used by bees. Mostly, it consists of 50% resin and vegetable balsam, 30% wax, 10% aromatic and essential oils, 5% pollen and 5% other substances, including organic debris [6]. Also, It is used as bactericidal, fungicidal, antivirulant anti-oxidant and anti-inflammatory [7]. The propolis extract contains amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, terpenes and caffeic acid. The propolis content of flavonoids provides it with anti-oxidant properties. So, it can catalysis of electron transport and scavenging free radicals [8] and the many properties of propolis are related with its content of flavonoids and phenolic acids [9].

Products from natural sources like propolis which has anti-oxidant activity may be benefit in the improvement of side effects of MSG. Wherefore, this work deals with the protective role of bee propolis against chromosomal aberrations and DNA damage in male albino mice after treatment with MSG.

MATERIALS AND METHODS

The present study used a MSG as white crystal powder from Allied Chemical Group, Egypt and a propolis as dark brown powder from Faculty of Agriculture, Ain Shams University, Egypt.

Forty male albino mice with (26-30 g) b.wt. divided into four groups (10 mice/group). First group was served as control group, Group 1 (10 mice) and the other three groups were treated as follows: 1 g/kg b.wt. of MSG, Group 2 (10 mice), 1 g/kg b.wt. of MSG in addition to 100 mg/kg b.wt. of propolis, Group 3 (10 mice) and 100 mg/kg b.wt. pf propolis, Group 4 (10 mice). The doses of MSG and propolis orally injected for two and four weeks.

Chromosomal aberrations

Chromosomes of the bone marrow cells examined to show the aberrations. Metaphase cells with well spreading (100 cells/mouse) examined in all groups- control and treated groups- after two and four weeks of treatment to calculate the all types of aberrations to show the effects of MSG and propolis [10].

Micronucleus test

Mouse bone marrow was prepared accordingly [11]. As an indicator of cell toxicity in bone marrow, the number of polychromatic erythrocytes (PCE) was counted by examining at least 200 erythrocytes (PCE + NCE) per animal under a microscope (e.g., × 1,000) with immersion oil. Then, the ratio of PCE to total erythrocytes was calculated as a percent. 2000 PCE/mouse were examined and the number of micronucleated polychromatic erythrocytes (MNPCE) was recorded and the percent of MNPCE to total PCE was calculated.

Comet assay

In the comet 100 cells/mouse were analyzed to detect the DNA migration. The comparing between tail comet length (µm) in all groups were done and evaluating the % DNA damage in tail length [12]. Each treated groups in all parameters were compared as mean and ± SD with the control group by independent samples T- test.

RESULTS

Chromosomal aberrations

Examination of bone marrow chromosomes of mice *Mus musculus* treated with MSG showed structural aberrations which were centromeric attenuation (Ca), deletion (D), ring form chromosome (R), fragment (F), centric fusion (Cf) and beaded chromosome (Bch). Also, the study found that MSG induced a change in number of bone marrow chromosomes which shown as polyploidy. These aberrations show in Figure 1.

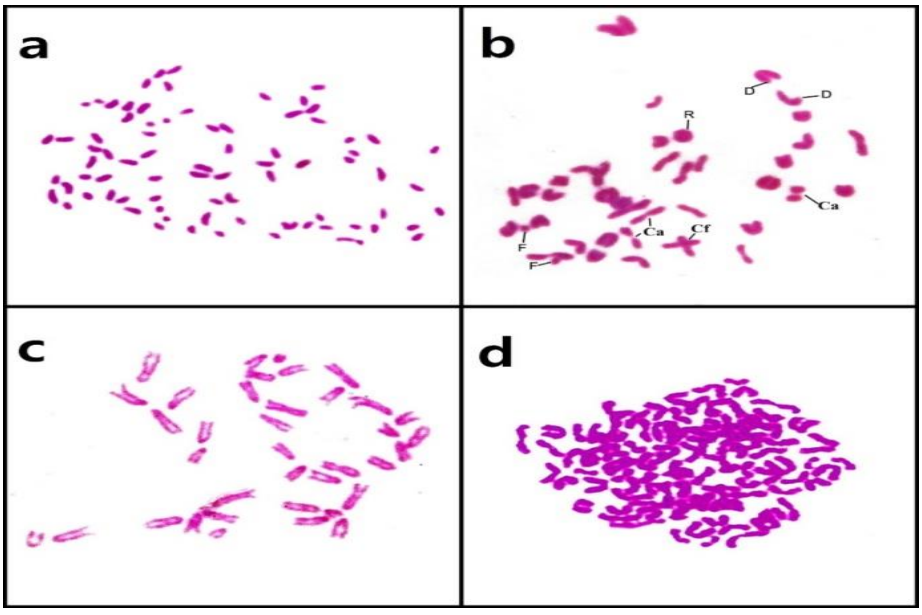

Figure 1: Photomicrograph of metaphase bone marrow chromosomes of mice showing a: Ca in all chromosomes, b: Ca, D, F and R chromosome c: Bch chromosomes and d: polyploidy (X: 2400)

Table 1 gives the mean ± SD of chromosomal aberrations of all treated mice and show the structural and numerical aberrations were significantly increased ($p<0.001$) compared with control group and these data show in Figure 2. Statistical analysis showed that chromosomal aberrations were significantly increased ($p<0.001$) by time. In the 2nd group (mice treated with 1 g/kg b.wt. MSG) after four weeks of treatment the Ca, D, R and F showed highly statistical mean compared to control and propolis group. While the treatment with MSG in addition to propolis showed decreasing in structural and numerical aberrations in bone marrow chromosomes compared with MSG group and control one.

Table 1: Mean and standard deviation of aberrations of bone marrow chromosomes of mice in all treated groups (Group 2 treated with 1 g/kg b.wt. of MSG, Group 3 treated with 1 g/kg b.wt. of MSG in addition to 100 mg/kg b.wt. of propolis and propolis group treated with 100 mg/kg b.wt.) and control group. Highly significant** $p<0.001$ – significant* $p<0.05$

Group	Time/Week	No. of metaphases cells	Structural aberrations						Numerical aberrations	Total aberrations
			Chromosomal aberrations				Chromatid aberrations			
			Centromeric attenuation (Ca)	Centric fusion (Cf)	Ring form (R)	Beaded chromosome (Bch)	Deletion (D)	Fragment (F)	Polyploidy (Pp)	
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control		500	1.2 ± 1.664	-	0.6 ± 0.55	-	0.8 ± 1.30	1 ± 1	-	3.6 ± 3.85
2	2	500	83.2 ± 8.04**	15.2 ± 3.63**	31.4 ± 5.46**	8.2 ± 2.86**	39 ± 8.43**	24.4 ± 2.3**	2.6 ± 1.14**	204 ± 16.49**
	4	500	92 ± 10.2**	19 ± 3.61**	37.2 ± 10.92**	10 ± 3.16**	47.6 ± 5.89**	30.4 ± 7.54**	6.4 ± 1.67**	242.6 ± 20.11**
3	2	500	50 ± 4.24**	9 ± 3.61**	9.6 ± 1.67**	1 ± 1	16 ± 2**	12.6 ± 1.34**	1.8 ± 1.48*	100 ± 6.16**
	4	500	54.8 ± 3.89**	7.6 ± 4.09**	11.2 ± 3.35**	4.4 ± 2.19**	34.8 ± 6.42**	20 ± 4.24**	4 ± 1.41**	136.8 ± 12.69**
Propolis		500	2.4 ± 1.14	0.4 ± 0.548	1.2 ± 1.3	-	1.2 ± 1.3	1.4 ± 1.14	-	6.6 ± 2.408

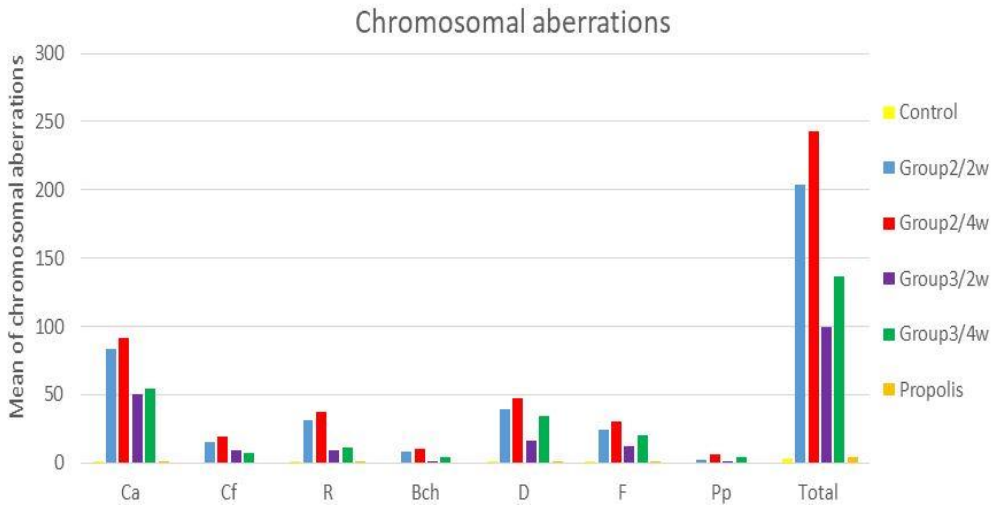

Figure 2: Histogram represents the mean of chromosomal aberrations (centromeric attenuation (Ca), centric fusion (Cf), ring form (R), beaded chromosome (Bch), deletion (D), fragment (F), polyploidy (Pp) and total aberrations (Total) in metaphases cells of male albino mice *Mus musculus* treated with MSG 1 g/kg (group 2), MSG 1 g/kg in addition to propolis (Group 3) after 2 and 4 weeks, propolis group and control group.

Micronucleus assay and cytotoxicity test

Bone marrow smear of control mice showing normochromatic erythrocytes (NCE) were small and polychromatic erythrocytes (PCE) were larger than NCE and bone marrow smear of treated mice showing micronuclei bluish in color in PCE as show in Figure 3.

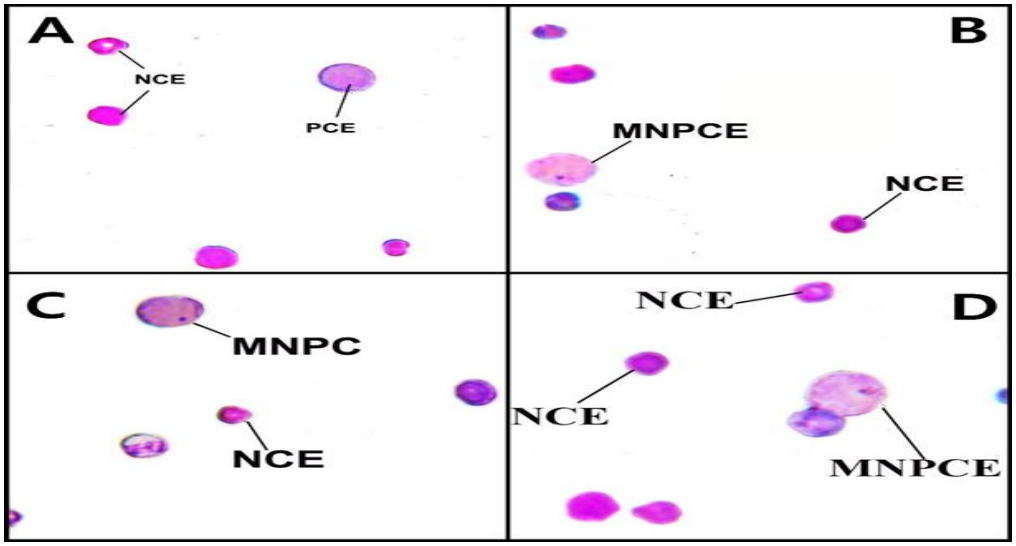

Figure 3: Photomicrograph of bone marrow smear showing A: Bone marrow smear of control male albino mouse Mus musculus and propolis group showing polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE), B,C and D: Bone marrow smear of treated mouse showing micronucleated polychromatic erythrocytes (MNPCE). X: 1500

The result of polychromic erythrocytes with micronucleus (MNPCEs) was summarized in Figure 4 as mean ± SD of for control and treated groups. Results of micronucleus assay in the present study showed that MSG treatment induced genotoxicity in bone marrow cells and the number of MNPCEs was gradually increased significantly ($p<0.001$) by time when compared to control one.

Also, cytotoxicity test showed that the ratio of PCEs was gradually decreased significantly ($p<0.001$) by time in the treated group with MSG when compared to the control one. The mice treated with 1 g/kg b.wt. of MSG after 4 weeks showed a high mean of MNPCEs and a low mean of ratio of PCE that used as an indication of toxicity in bone marrow. While the treatment with MSG in addition to propolis showed significantly decreasing ($p<0.05$) in MNPCE and improved ($p<0.05$) the ratio of PCE in bone marrow compared to control one.

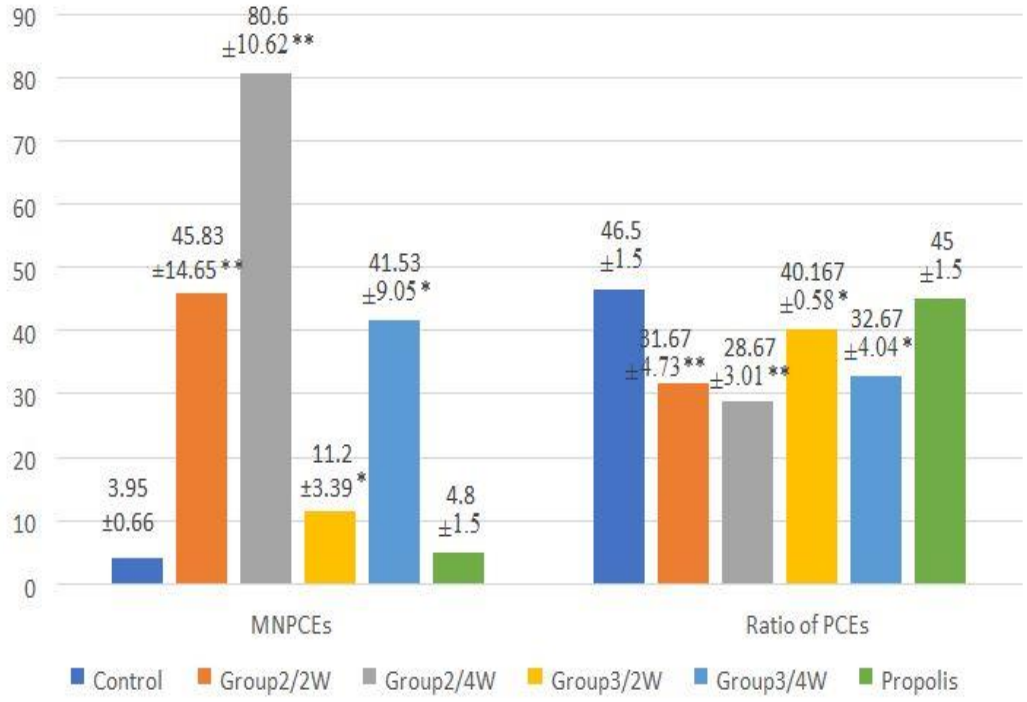

Figure 4: Histogram represent the relationship of mean micronucleated polychromatic erythrocytes (MNPCEs) and mean of ratio polychromatic erythrocytes (ratio of PCEs) in control group and all treated groups 1 g/kg b.wt. MSG (Group 2), 1 g/kg b.wt MSG in addition to propolis (Group 3) and propolis group after 2 and 4 weeks (2W and 4W). The data expressed as mean ± SD, Highly Significant ** p<0.001 - Significant * p<0.05 when compared to control group.

Comet assay

Mice treated with MSG showed a high degree of DNA damage which clarified by the migration of fragmented DNA (tailed) through the images of single cell gel electrophoresis as show in Figure 5.

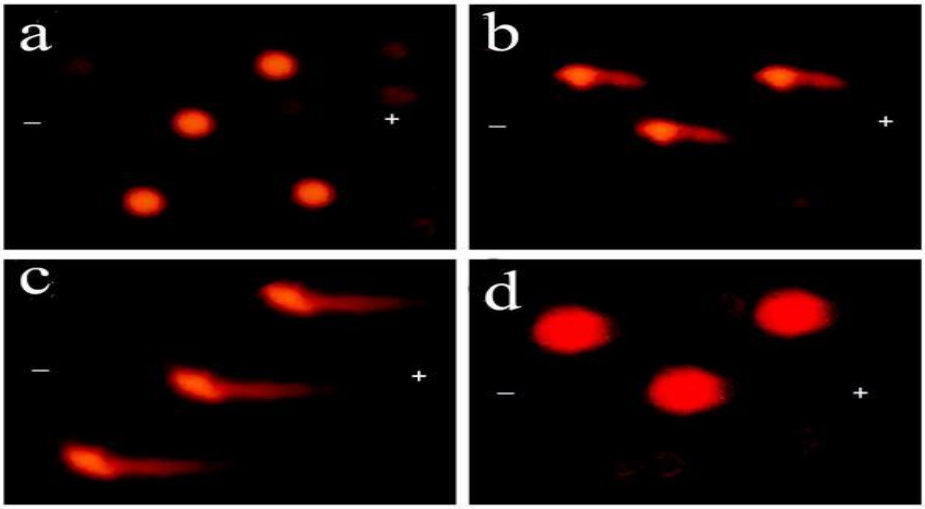

Figure 5: Photomicrograph of comet assay showing a: Control group showing no or minimum DNA migration, b: Group 2 which treated with 1 g/kg b.wt. of monosodium glutamate (MSG) after 2 weeks of treatment showing the most extensive DNA migration (very long tails), c: Group 2 which treated with 1 g/kg b.wt. of MSG after 4 weeks of treatment showing the most extensive DNA migration (very long tails), d: Group 3 which treated with 1 g/kg b.wt. of MSG in addition to propolis after 2&4 weeks of treatment showing slight DNA migration (short tails). The symbols '_' and '+' represent cathode and anode, respectively, during the electrophoresis. (X: 1500).

The current results of comet assay showed that treatment with MSG significantly increased (p<0.001) the mean of DNA damage in liver cells of the treated mice in time dependent manner. Statistical analysis showed that the mean of DNA damage scored by comet assay parameter was highly significant increased (p<0.001) in Group 2 (1 g / kg b.wt. of MSG) after 4 weeks of treatment. While the treatment with MSG in addition to propolis statistical decreased (p<0.05) the DNA damage compared to control one as shows in Figure 6.

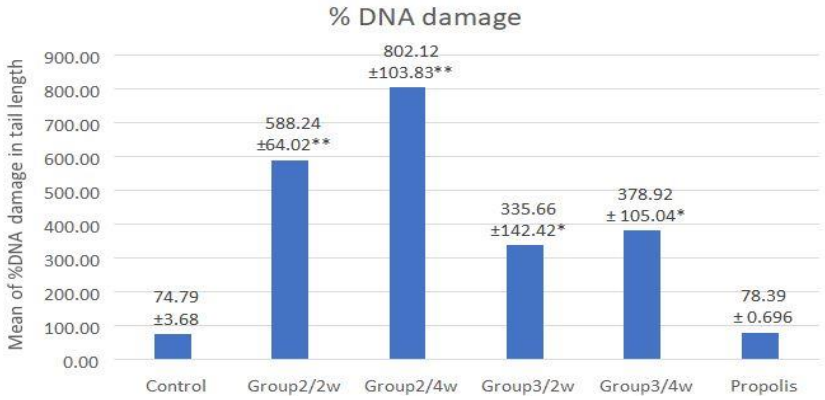

Figure 6: Histogram represents the relationship of mean % DNA damage in tail length control group and all treated groups: 1 g/kg b.wt. MSG (Group 2), 1 g/kg b.wt. MSG in addition to propolis (Group 3) and propolis group after 2 and 4 weeks (2W and 4W). The data expressed as mean ± SD, Highly Significant ** p<0.001 - Significant * p<0.05 when compared to control group.

DISCUSSION

In this study, the possible genotoxic effect of MSG was investigated by using chromosomal aberrations and micronucleus in mice bone marrow cells as well as comet assay in mice liver cells. These tests most used to measure the chemicals mutagen and carcinogen [13,14].

The result of chromosomal aberrations in this study showed that the Ca and D were the most common types demonstrated after MSG treatment and the maximum of these occurred after four weeks of treatment. Occurrence of these aberrations would indicate that the chemical possibly acted after chromosome duplication at the G2 phase of the cell cycle [15]. MSG at dose a 1000 mg/kg b.wt. Induces a significant increase in the chromosomal aberrations and this increase depends by time [16]. Our results of chromosomal aberrations agree with author who revealed that MSG induces bone marrow chromosomal aberrations in rats. These aberrations are structural aberrations like chromosomal gap, chromatid gap, D, R, Cf, F and Ca and numerical aberrations in the form of polyploidy. Ca is the most common aberration and is considered as an authenticated biomarker of cancer risk in humans [17].

The possibility of MSG clastogenic and aneugenic can be detected by the micronucleus assay. MSG increases the MNPCE in lymphocytes [18]. Micronucleus assay revealed that MSG induced highly significant ($p<0.001$) in MNPCEs in treated group (1 g/kg b.wt.) after two and four weeks and MNPCEs gradually increased by time. Also, the ratio of PCE was gradually decreased by time in group treated with MSG compared with control group and increased by time in group treated with MSG in addition to propolis compared with group treated with MSG only. The higher doses of MSG (500 and 1000 mg/kg b.wt.) show a significant increase in the micronuclei in PCE and change in the ratio of PCE after 48 and 72 hours of treatment compared with the control [16]. The free radicals and DNA alkylation which generated from MSG cause chromosome damage and produce mutation [5]. Group 2 (1 g/kg b.wt. of MSG) after four weeks of treatment showed the maximum damage in DNA compared to control. All doses of MSG (250, 500, 1000, 2000, 4000 and 8000 mg/ml) show a significant increase in parameters of comet assay (tail intensity, tail length and tail moment) and these increases are dose dependent [18].

These effects could be attributed to reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radicals and superoxide anion [1]. When ROS overcome the defense system of the cell, and redox homeostasis is altered, the result is oxidative stress [19]. Excess generation of ROS in cells is known to damage DNA, lipids, and proteins resulting in several biological effects, ranging from alterations in signal transduction, gene expression, mutagenesis, apoptosis, structural and numerical chromosomal aberrations in this study. Oxidants by MSG can directly attack the backbone of a protein to cause fragmentation [20-23]. MSG induces lipid peroxidation in several organs [1], the end-products of lipid peroxidation like a malondialdehyde (MDA) react with deoxyadenosine and deoxyguanosine in DNA and forming DNA adducts. So, it may be mutagen or carcinogen [24].

The propolis has a therapeutic activities depend on its flavonoids. these flavonoids able to scavenge the free radicals and may binding to heavy metal ions to suppress the formation of free radicals [25], as in this study the MSG in addition to propolis showed a significant decreased in the damage of bone marrow chromosomes and DNA. Moreover, experimental studies showed that propolis exerts protective effects on different tissues [26]. *In vitro* tests propolis induces cell cycle arrest, apoptosis and reduction of expression of growth and transcription factors, including NF-κB. Notably, caffeic acid phenethyl ester down-regulates the mdr-1 gene, and is considered responsible for the resistance of cancer cells to chemotherapeutic agents [27]. *In vivo* studies on mice, propolis inhibits 4 - (methyl nitrosamino) - 1 - (3 - pyridyl) – 1 – butanone - induced tumorigenesis [28]. Propolis is a substance riches with essential elements like Zn^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+} and Ca^{2+} that may responsible for reactivating antioxidant enzymes [29].

CONCLUSION

Results of this study concluded that monosodium glutamate induced a significantly increased chromosomal aberrations, micronucleus in bone marrow and DNA damage, therefore, the using of MSG should be restricted to a very narrow range border owing to its harmful genotoxic effect. Also, the study found that propolis plays a therapeutic role in reducing these effects because it is an antioxidant.

REFERENCES

[1]. Farombi, E.O., Onyema, O.O., Monosodium glutamate-induced oxidative damage and genotoxicity in the rat: Modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Toxicol, **2006.** 25:251-259.

[2]. Husarova, V., Ostatnikova, D., Monosodium Glutamate Toxic Effects and Their Implications for Human Intake: A Review. JMED, **2013.** p. 1-12

[3]. Gill, S.S., Mueller, R.W., McGuire, P.F., et al. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. Toxicol Pathol, **2000.** 28:277-284.

[4].Collison, K.S., Effect of Dietary Monosodium Glutamate on HFCS-Induced Hepatic Steatosis: Expression Profiles in the Liver and Visceral Fat. Obesity, **2010.** 18:1122-1134.

[5].Ismail, N.H., Assessment of DNA damage in testes from young Wistar male rat treated with monosodium glutamate. Life Sci J, **2012.** 9:930-939.

[6]. Viuda, M.M., Ruiz, N.Y., Fernández, L.J., et al. Functional properties of honey, propolis, and royal jelly. J Food Sci, **2008.** 73:117-124.

[7]. Pujirahayu,N., Ritonga, H., Satya, A.L., et al. Antibacterial activity of oil extract of Trigona propolis. Int J Pharm Pharm Sci, **2015.** 7:419-422.

[8]. Russo, A., Longo, R., Vanella, A., Antioxidant activity of propolis: Role of caffeic acid phenethyl ester and galangin. Fitoterapia, **2002.** 73: 21-29.

[9]. Farooqui, T., Beneficial effects of propolis on human health and neurological diseases. Front Biosci, **2012.** E4:779-793.

[10]. Julian, P.R., Dean, B.J., Galloway, S., et al. Mammalian *in vivo* cytogenetic assays analysis of chromosome aberrations in bone marrow cells. Mutat Res Toxicol, **1987.** 189:157-165.

[11]. Kasamoto, S., Masumori, S., Hayashi, M., *In vivo* micronucleus assay in mouse bone marrow and peripheral. Blood, **2013.**1044:179-189.

[12]. Singh, N.P., McCoy,M.T., Tice, R.R., et al. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res, **1988.** 175:184-191.

[13]. Chandirasekar, R., Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. Mutat Res Genet Toxicol Environ Mutagen, **2014.** 767:21-27.

[14].Huerta, I., Genotoxic evaluation of five Angiotesin II receptor blockers: *In vivo* and *in vitro* micronucleus assay. Mutat Res Genet Toxicol Environ Mutagen, **2014.** 767:1-7.

[15]. Biswas, S.J., Pathak, S., Khuda-Bukhsh, A.R., Assessment of the genotoxic and cytotoxic potential of an anti-epileptic drug, phenobarbital, in mice: A time course study. `Mutat Res Genet Toxicol Environ Mutagen,**2004.** 563:1-11.

[16]. Dadhaniya, D.R., Shama, K.P., Prakash, U.D., et al. Toxicological Studies of Monosodium Glutamate-A Food Additive. Int J Pharm Chem Biol Sci, **2018.** 8:305-318.

[17]. Mahmoud, M., Hegazy, E., In Submandibular Salivary Glands and Prevent. Egypt Dent J, **2018.** 64:733-746.

[18]. Ataseven, N., Yüzbasıoglu, D., Keskin, A.C., et al. Genotoxicity of monosodium glutamate. Food Chem Toxicol, **2016**. 91:8-18.

[19]. Bonnefont, R.D., Bastard, J.P., Jaudon, M.C., Consequences of the Diabetic Status on the Oxidant/Antioxidant Balance. Diabetes Metab, **2000.** 26 (3):163-76.

[20]. Rollet, L.E., Grange, M.J., Elbim, C., et al. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. Free Radic Biol Med, **1998.** 24.563-572.

[21]. Kannan, K., Jain, S.K., Oxidative stress and apoptosis. Pathophysiology, **2020.** 7:153-163.

[22]. Martinaud, A., Mercier, Y., Marinova, P., et al. Comparison of Oxidative Processes on Myofibrillar Proteins from Beef during Maturation and by Different Model Oxidation Systems. J Agric Food Chem, **1997.** 45:2481-2487.

[23]. Morzel, M., Gatellier, P., Sayd,T., et al., Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. Meat Sci, **2006.** 73:536-543.

[24]. Marnett, L.J., Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res Fundam Mol Mech Mutagen, **1999.** 424:83-95.

[25]. Cavallini, S.N., Bindoli, A., Comparative evaluation of antiperoxidative action of silymarin and other flavonoids. Pharmacol Res Commun, **1978.**133-136.

[26]. Kosalec, I., Pepeljnjak, S., Bakmaz, M., et al. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Acta Pharm, **2005.** 55:423-430.

[27]. Wu, J., Caffeic acid phenethyl ester (CAPE), derived from a honeybee product propolis, exhibits a diversity of anti-tumor effects in pre-clinical models of human breast cancer. Cancer Lett, **2011.** 308:43-53.

[28]. Sugimoto, Y., Inhibitory effects of propolis granular A. P. C on 4- (methylnitrosamino)-1- (3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. Cancer Lett, **2003.** 193:155-159.

[29]. Haro, A., López, A.I., Lisbona, F., et al. Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. J Agric Food Chem, **2000.** 48 (11):5715-5722.