The Egyptian Journal of Hospital Medicine (Jul. 2016) Vol. 64, Page 311- 318

Role of Quercetin against 2-Butoxyethanol Induced Micronucleus and Chromosome Aberrations in Mice

Zeinab Eid Madboly Hanafy [1], Hassan Abd El sattar Eldawy [2], Karima Fathi Mahrous, [3] Asmaa Kutb Mohamed[1]

[1] Zoology Department, Faculty of Science, Al–Azhar University (Girls), [2] Radiation biology Department, National Center for Radiation Research and Technology, [3] Cell biology Department,National research center.

ABSTRACT

EGBE is also known as 2-butoxyethanol (2BE). EGBE is widely used as a solvent in various applications, such as in surface coatings, spray lacquer, quick-dry lacquers, enamels, varnishes, varnish removers, latex paint, metal cleaners, and in commercially available cleaning products. 2-Butoxyethanol causes cellular damage via formation of reactive oxygen species. Quercetin, a flavonol group of plant flavonoid, has generated interest because of its potential antioxidant, anti-proliferative, chemoprotective and anti-inflammatory properties. Quercetin exhibited significant antimutagenic and DNA-protective effects against oxidative damage due to the presence of hydroxyl groups in the molecule; it is considered an excellent free radical scavenging antioxidant owing to the high number of hydroxyl groups.

Materials and Methods: 2BE was given orally to male mice for seven days at two doses (450µl &900µl/kg b.w.). Quercetin was dissolved in corn oil. The animals in positive control group were treated with only corn oil. Quercetin (20 mg/kg b.w.) was administered orally for 7 days prior to 7 days treatment of 2BE for the two doses. Quercetin (20 mg/kg b.w.) was also administered for 14 days (7 days before and 7 days during the period of treatment with two doses of 2BE).

Result: the two doses of 2BE induced both micronuclei (2.88% and 12.77%) and chromosomal aberration in (50% & 72.8%, respectively) of cells. Quercetin administration lowered the frequency of micronuclei and chromosome aberration.

Conclusion: These effects of quercetin are to be taken into consideration while evaluating the possible use of quercetin as a protective agent.

Key words: Chromosome aberration, Micronucleus, 2-Butoxyethanol, Quercetin

INTRODUCTION

About 50% of synthesized organic solvents (OSs) are employed for the production of paints and thinners. 2-Butoxyethanol, Xylene, toleune, styrene, ethylbenzne , acetone and methyl ethylketone are some of most frequently and quantitatively represented solvents in the composition of paints [1]. It is well documented that several OSs are potent carcinogens among population at risk. Their genetic effects have important implications for cancer induction[2]. EGBE is also known as 2-butoxyethanol. EGBE is widely used as a solvent in various applications, such as in surface coatings, spray lacquer, quick-dry lacquers, enamels, varnishes, varnish removers, latex paint, metal cleaners, and in commercially available cleaning products [3]. The toxic effect of 2-butoxyethanol is caused by the formation of reactive oxygen species (ROS). There are several studies reporting that 2-butoxyethanol causes cellular damage via formation of ROS. ROS are believed to cause lipid peroxidation resulting in damage to biological membranes [4].

Quercetin (QUR) is a polyphenol (3, 3', 4', 5, 7-pentahydroxyflavone). QUR found in vegetables and fruits in the form of a glycoside (with an exceptionally high concentration in onions, apples, tea and broccoli) [5].Several studies indicate that QUR has multiple beneficial effects [6]. Among the many attributes of QUR are its antioxidative[7]; anti-inflammation [8]and anti-apoptosis [9]; properties. Furthermore, there is reason to believe that QUR can shield the liver from damage brought about by hepatotoxins [10].

MATERIALS AND METHODS

1. Chemicals

2-Butoxyethanol (2BE), and Quercetin (3,3_,4_,5,7-pentahydroxy flavone) were purchased from Alpha Aesar, Germany. Colchicine and Giemsa stain were purchased from Sigma Chemicals Co.

The chemical solutions were freshly prepared prior to experimentation as followes:

Quercetin: 26 mg was dissolved in 5ml corn

Received: 02/05/2016
Accepted: 10/05/2016

DOI : 10.12816/0029023

oil. 2-Butoxyethanol suspension: 11.7 ml were added to 88.3 ml water.

2.2. Experimental animal

One hundred and twenty Male mice between 8 and 10 weeks old, weighing 26±2 g were divided into 12 groups (10 animals per group), maintained at room temperature at 28±4◦C with 12 h dark and 12 h. light cycle were used for the study. Standard food pellets and water were provided *ad libitum.*

2.3. Dose and treatment

One hundred and twenty male mice were divided into 12 groups (10 animals/group). Each animal was orally administered the test solution according to the following scheme:

G1a: Water control for 7 days.**G1b**: Water control for 14 days.**G2a:** 7 day corn oil (*0.1 ml daily for 7 days*)**.G2b:** 14 day corn oil (*0.1 ml daily for 14 days*)**.G3a**: 7 day quercetin : *0.1ml of the suspention daily for 7 days*)**.G3b: 14 day quercetin :** *0.1ml of the suspention daily for 14 days*)**. G4:**2butoxyethanol (450µl /kg b.w.)[12] : 0.1 ml *daily for 7 days.***G5:** 2butoxyethanol (900µl /kg b.w.)[12] : *0.2 ml daily for 7 days*

G6a: quercetin was administered for 7 days before to 7 days treatment of 2BE (450µl µl/kg b.w.). **G6b:** quercetin was administered for 7 days before to 7 days treatment of 2BE (450µl µl/kg b.w.) and 7 days during 2BE treatment.**G7a:** quercetin was administered for 7 days before 7 days treatment of 2BE (900µl µl/kg b.w.).**G7b:** quercetin was administered for 7 days before to 7 days treatment of 2BE (900µl µl/kg b.w.) and 7 days during 2BE treatment

4. Micronucleus (MN) assay

At the end of experiment, The MN slides were prepared as described by **Schmid [13].**

The bone marrow was extracted and smear was prepared, air dried and fixed in absolute methanol for 10 min and stained with 5% buffered Giemsa (pH 7.0) in the following day. Polychromatic erythrocytes were scored for micronuclei under the microscope.

5. Chromosome aberration (CA) assay

The CA assay was carried out as described by **Yosida and Amano [14]**. Experimental animals were injected with 2mg/kg b.w. of colchicine 1.5 h prior to sacrifice following 24 h and 48 h of the treatmen of test chemicals. Bone marrow cells were collected by flushing with 0.56% KCl (pre-warmed at 37 ◦C) from femur bone and incubated for 20 min at 37 ◦C. The material was centrifuged at 1000rpm for 5min, fixed in freshly prepared aceto-methanol (acetic acid and methanol in the ratio 1:3, v/v) followed by refrigeration for 30 min. The material was centrifuged and re-suspended in aceto-methanol. The slides were prepared by dropping the sample on chilled slides and run over the flame. Staining was done in 5% buffered Giemsa stain (pH 7.0) after 24h, air dried and covered with cover slips. 50 well spread metaphase plates were studied per animal (5 animals/group).

6. Statistical analysis

The data obtained in the present work were represented in tables as mean ±standard error. Statistical analysis was carried out using (Statistical Package for Social Science) (SPSS) software version 20 for windows, significant differences among groups were evaluated using one-way analysis of variance (one-way ANOVA).

RESULTS

1. Effect on micronucleus frequency

Table (1) shows the results of micronucleus (MN) analysis following quercetin alone and/or 2BE. The percentage of cells with micronuclei (fig.,1) following two doses of 2 BE (450µl/kg b.wt. and 900µl/kg b.wt.) were 2.88% and 12.77%, respectively these results were significant as compared to control group ($P<0.05$)**.**Treatment of quercetin before 2BE reduced the frequency of cells with MN at both two doses of 2BE at 7 days (2.08% & 9.87%) and 14 days (1.19% &6.12%)

2. Effect on chromosomal aberration frequency

The detailed data of chromosomal aberration **(**CA) analysis are shown in Table (2&3). Quercetin induced non-significant increase in the frequency of abnormal cells with CA ($P < 0.05$). 2-butoxyethanol induced both structural and numerical aberrations (fig., 1,2&3) at the two doses (450µl/kg b.wt. and 900µl/kg b.wt.), the percentage of abnormal cells recorded were (50% & 72.8%, respectively). This percentage decreased after 7 days treatment with quercetin

(22% & 33.2%), respectively for the two doses of 2BE and (9.6% &16.4%) after 14 days. Quercetin protected against the effect of 2BE by reducing the percentage of aberrant cells. The highest level of protection was observed at 14 days.

DISCUSSION

The cytogenetic effects of exposure to Organic solvents is due to their ability to make oxidative stress. Oxidative stress (OS) is caused by increased exposure to oxidants and / or decreased antioxidants capacities. It is widely recognized as a central feature of many diseases [15].Exposure to OSs can also lead to mood disorders, with depression, irritability and fatigue being common symptoms. Peripheral neurotoxicity usually results in paresthesia, tremors and diminished fine and gross motor movements. Volatile organic compounds have been implicated also in kidney damage [16].

Our study aimed mainly to evaluate the cytogenetic changes associated with exposure to 2-butoxyehanol compound by oral ingestion. 2-Butoxyethanol, a chemical solvent that has been shown to induce hemangiosarcomas mainly in livers of male mice [17], with some occurrence in the bone marrow (BM) and spleen after inhalation exposure [3].

Exposure 2-butoxyethanol resulted in genetic instability characterized by a delay in cell division and resulted in a significantly higher level of chromosomal aberrations and a higher percentage of aberrant cells in than in their matched controls, they had a significantly higher frequency of breaks, polyploid and endoreduplicated cells which can result in the development of specific congenital anomalies [18]. The increase was concentration dependent. The detection of polyploidy (which corresponds to an exact multiple of the haploid number of chromosomes) and endoreduplication (in which at mitosis the chromosome consists of 2 doubled chromatids instead of 2 single chromatids) resulting from exposure to 2-butoxyethanol, indicating defects in the Gl/S and G2/M checkpoints of the cell cycle [19].

El-Zein *et al* [20]suggested that exposure to EGME (2-BE) in utero could result in terminal chromosome rearrangements and shortening of telomere length, leading to the observed dysmorphic features and idiopathic mental retardation **Hoyos-Giraldo,** demonstrated a significant CAs frequency increase in painters exposed to organic solvents [21]. Organic solvent extracts induced structural CAs dose-dependently, including chromatid and chromosome gaps; chromatid breaks; chromatid exchanges; fragmentation; chromosome breaks; chromosome exchange **[22].**

Therefore our study gives a better cancer risk insight associated with organic solvents exposure in order to avoid future cancers. Our CAs frequency increase results are consistent with previous studies, in out-door and in-door painters occupationally exposed to organic solvents[21].

The mode of action of 2BE involves the hemolysis of RBCs, since it induced haemolytic anaemia in rats at dose of 450 mg/kg [23]. Hemolysis results in iron deposition which causes reactive oxygen species production resulting in oxidative damage, these factors may then produce oxidative DNA damage **[12].**

The percentage of cells with micronuclei was significantly higher in animals treated with 2 doses of 2BE than control. The increase was concentration dependent. This suggests that it has a role in the process of cell division since micronuclei were reported as due to chromosomal fragmentation and or whole chromosome lagging behind in anaphase [24]. This may lead to apoptosis [25]. Apoptosis of polychromatic erythrocytes leads to hemolytic anemia [23] and liberation of iron which production of reactive oxygen species and increased oxidative stress [12]

Quercetin displayed various cancer chemoprotective and chemopreventive effects **[26].**The mechanism of pharmacological action was related at least in part to the antioxidant activity of quercetin [27]**.** Quercetin induced neither micronucleated erythrocytes nor DNA damage [28]**.**

Growing evidences suggest that flavonoids (in particular, resveratrol and quercetin) may contribute to chromatin remodeling and thus interfere with epigenetic alterations that are important in cancer progression. Chromatin is remodeled by chemical modifications of DNA and histones, such as DNA methylation and multiple histone modifications, such as

methylation, phosphorylation, acetylation, sumoylation and ubiquitination; for example, resveratrol activates sirtuin (SIRT)-1, a member of histone deacetylase (HDAC) family, which plays key roles in cell survival and apoptosis [29].
The decrease in mitomycin C induced MN and CA frequency by the action of quercetin is a phenomenon of attenuation (antioxidant activity and/or stimulation of DNA repairing process) or synergism (enhanced apoptotic process resulting in lower recovery of viable mutants) **[30].** It has been reported that quercetin acts through the mitochondrial pathway to induce apoptosis in cancer cells **[31].**

Quercetin exhibited antimutagenic and DNA-protective effects against oxidative damage due to the presence of hydroxyl groups in the molecule, it is considered an excellent free radical scavenging antioxidant owing to the high number of hydroxyl groups [32]. Quercetin is well known as a chelating agent that inactivates the metal iron responsible for the generation of reactive oxygen species[33].

CONCLUSION

People who have to use organic solvents as a part of their daily work are experiencing the effects of 2-butoxyethanol exposure. Quercetin was found to be very excellent protective plant extract antioxidants protect against free radicals formed as a result of metabolism of 2-butoxyethanol, these free radicals that harm genetic material leading to dangerous diseases on the long duration. It is found to reduce the frequency of chromosome and chromatid type aberrations and they limit the number of micro nucleated cells. The protective action of quercetin is more effective at 14 days than 7 days.

REFERENCES

1- **Costa C, Pasquale R and Barbro M C (2005):** In vitro evaluation of oxidative damagefrom organic solvent vapors on human skin.Toxicol in Vitro, 20:324-331.

2- **Safty A E, Metwally F M, Samir A M, Shahawy A E, and Raouf E A (2015):** Studying the effect of antioxidants on cytogenetic manifestations of solvent exposure in the paint industry. Toxicology and Industrial Health, 31(12):1087–1094.

3- **National Toxicology Program (NTP), (2000):** Toxicology and carcinogenesis studies of 2-butoxyethanol (CAS NO. 111-76-2) in F344/N rats and B6C3F1 mice (inhalation studies). Natl. Toxicol. Program Tech. Rep. Ser., 484:1–290.

4- **Stacy M C, Lisa M K and James E K (2006):** Mechanisms of 2-Butoxyethanol–Induced Hemangiosarcomas. Toxicol. Sci., 92(2):378-86.

5- **Alrawaiq N S and Abdullah A (2014):** A Review of Flavonoid Quercetin: Metabolism, Bioactivity and Antioxidant Properties. International Journal of Pharm Tech Research, 6(3): 933-941.

6- **Chirumbolo S (2012):** Role of quercetin in vascular physiology. Canadian journal of physiology and pharmacology, 90(12):1652-1657.

7- **Nabavi S F, Nabavi S M, Mirzaei M and Moghaddam A H (2012):** Protective effect of quercetin against sodium fluoride induced oxidative stress in rat's heart. Food & Function, 3(4):437-441.

8- **Hwang I K, Lee C H and Yoo K Y (2009):** Neuroprotective effects of onion extract and quercetin against ischemic neuronal damage in the gerbil hippocampus. J. Med. Food, 12:990-995.

9- **Cheng Y F, Zhu G Q and Wang M (2009):** Involvement of ubiquitin proteasome system in protective mechanisms of Puerarin to apoptosis. Neuro science research, 63(1):52-58.

10- **Ghosh A, Mandal A K, Sarkar S, Panda S and Das N (2009):** Nanoencapsulation of quercetin enhances its dietary efficacy in combating arsenic-induced oxidative damage in liver and brain of rats. Life Sciences, 84(3):75-80.

11- **Patil S L, Rao N B, Somashekarappa H M and Rajashekhar K P (2014):** Antigenotoxic Potential of Rutin and Quercetin in Swiss Mice Exposed to Gamma Radiation. Biomed J., 37 (5):1-9.

12-Siesky AM, Kamendulis L M and Klaunig J E (2002): Hepatic effects of 2-butoxyethanol in rodents. Toxicol. Sci., 70: 252-260.

13-Schmid W (1975): The micronucleus test. Mutat. Res., 31:9-15.

14-Yosida T M and Amano K (1965): Autosomal polymorphism in laboratory bred and wild Norway rats Rattus norvegicus found in Misima. Chromosoma, 16: 658-667.

15- **Halifeoglu I, Canatan H, Ustundag B, Ihan N and Inanc F (2000):** Effect of thinner inhalation on lipid peroxidation and some antioxidant enzymes of people working with paint thinner. Cell Biochem. Function, 18(4):263-267.

16- **Verplanke A J and Herber R F (1998):** Effects on the kidney of occupational exposure to styrene. Int. Arch. Occup. Environ. Health, 71:47-52.

17- **Nyska A, Haseman J K, Kohen R and Maronpot R R (2004):** Association of liver hemangiosarcoma and secondary iron overload in B6C3F1 mice--the National Toxicology Program experience. Toxicol.Pathol., 32: 222-228.

18- **El-Zein R A, Abdel-Rahman S Z, Morris D L and Legator M S (2002):** Exposure to ethylene glycol

monomethyl ether: clinical and cytogenetic findings.Arch Environ Health, 57:371–376.
19- **Kirsch-Volders M, Vanhauwaert A and De Boeck M (2002):** Importance of detecting numerical versus structural chromosome aberrations. Mutat Res., 504(1-2):137-48.
20- **El-Zein R , Albrecht T, Knutson E, Legator M S and Abdel-Rahman S Z (2007):** Reduction in Telomere Length in Individuals Exposed In Utero to Glycol Ether. Archives of Environmental & Occupational Health, 62: 3.
21- **Hoyos-Giraldo L S, Carvajal S, Cajas-Salazar N, Ruíz M and Sánchez-Gómez A (2009):** Chromosome aberrations in workers exposed to organic solvents: Influence of polymorphisms in xenobioticmetabolism and DNA repair genes, Mutat. Res., 18, and 666(1-2): 8-15.
22- **Matsuoka A, Haishima Y, Hasegawa C, Matsuda Y and Tsuchiya T (2008):** Organic-solvent extraction of model biomaterialsfor use in the in vitro chromosome aberration test. J Biomed Mater Res A., 86(1):13-22.
23- Kenyon M O, Coffing S L, Ackerman J I, Gunther W C, Dertinger S D, Criswell K and Dobo K L (2015): Compensatory erythropoiesis has no impact on the outcome of the *in vivo Pig-a* mutation assay in rats following treatment with the haemolytic agent 2 butoxyethanol.*Mutagenesis*, 30(3):325-334.
24- **Albertini R J, Aderson D, Dauglas G R , Hagmar L, Hemminki K, Merlo F, Natarajan A T , Norppa H , Shuker D EG, Tice R ,Waters M D and Aitio A (2000):** IPCS guidelines for the monitoring of genotoxic effects of carcinogens in human, Mutat. Res., 463: 111–172.
25- **Ferguson L R, Whiteside G, Holdaway K M and Baguley B C (1996):** Application of fluorescence in situ hybridisation to studying the relationship between cytotoxicity, chromosomal aberrations and changes in chromosome number after treatment with the topoisomerase II inhibitor, amsacrine, Environ. Mol. Mutagen, 27: 255–262.
26- **Russo M, Spagnuolo C, Tedesco I, Bilotto S and Russo GL (2012):** The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochem Pharmacol., **83**:6–15.
27- **Jung J H, Kang J I and Kim H S (2012):** Effect of quercetin on impaired immune function in mice exposed to irradiation. Nutr. Res. Pract., 6: 301–307.
28- **Tieppo J, Vercelino R, Dias A S, Silva Vaz M F, Silveira T R, Marroni CA, Marroni N P, Henriques J A P and Picada J N (2007):** Evaluation of the protective effects of quercetin in the hepatopulmonary syndrome. Food and Chemical Toxicology, 45: 1140-1146.
29-Borra M T, Smith B C and Denu J M (2005): Mechanism of human SIRT1 activation by resveratrol. Journal of Biological Chemistry, 280(17):17187–17195.
30-Mazumdar M, Giri S and Giri A (2011): Role of quercetin on mitomycin C induced genotoxicity: Analysis of micronucleus and chromosome aberrations in vivo. Mutation Research, 721: 147–152.
31- **Vidya P R, Senthil M R and Maitreyi S (2010):** The flavonoid quercetin induces cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells through p53 induction and NF-kappaB inhibition. Eur. J. Pharmacol., 649:84-91.
32- **Valentova K P, Rybkova Z, Kˇrižan J, Malachova K and Kˇrena V (2016):** (Anti) mutagenic and immunomodulatory properties of quercetin glycosides. J Sci. Food Agric., 96: 1492–1499.
33- **Kaeko M, Yuki M, Mami I, Tojiro T, Sayuri M and Junji T (2004):** Quercetin- 4_-glucoside is more potent than quercetin-3-glucoside in protection of rat intestinal mucosa homogenates against iron-induced lipid peroxidation. *J Agric Food Chem*., 52:1907–1912.

Table (1): Percent of cells with micronuclei and mean number of micronucleated cells ±Standard error in control and treated groups (The total number of scored cells is 10000)

Groups	% of cells with micronuclei	Mean±SE
Control(G1)	0.32	6.4±0.2
Oil(G2a)	0.46	9.2±0.37 a
Oil(G2b)	0.38	7.2±0.37 a
Quercetin 7 days(G3a)	0.55	11±0.44 a
Quercetin 14 days(G3b)	0.63	12.6±0.4 a
2-butoxyethanol (450 ul)(G4)	2.88	57.6±0.5 a
Quercetin 7+ 2BE (450)(G6a)	2.08	41.6±0.5 abd
Quercetin 14+ 2BE (450)(G6b)	1.19	23.8±0.37 abd
2-butoxyethanol (900 ul)(G5)	12.77	225.4±1.5 a
Quercetin 7+ 2BE (900)(G7a)	9.87	197.4±0.5 acd
Quercetin14+ 2BE (900)(G7b)	6.12	122.4±0.5 acd

a: significantly different as compared with control, b: significantly different as compared with 2-butoxyethanol (450µl) ,c: significantly different as compared with 2-butoxyethanol (900µl),d: significantly different as compared with the corresponding group at the other time interval

Table (2): Chromosomal aberration frequencies in bone marrow cells of mice treated with quercetin for seven days with different doses of 2-butoxyethanol.

Groups	No. of scored cells	Abnormal cells (%)	Cells with more than one aberration (%)	Chromatid type aberrations (%)		Chromosome type aberrations (%)					Total structural aberrations (%)	Numerical aberrations (%)		Total num. aberr. (%)	Total aberration Excluding g. (%)
				Chd.g.	Chd. br	Chs. g	D	F	CA	Ring		Polyploidy	Aneuploidy		
Control (G1a)	250	1.0± 0.0	0.0± 0.0	0.4± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.6± 0.24	0.0± 0.0	0.0± 0.0	1.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.6± 0.24
Oil (G2a)	250	1.4± 0.24	0.0± 0.0	0.6± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 0.2	0.0± 0.0	0.0± 0.0	1.4± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 0.2
Quercein (G3a)	250	1.6± 0.24	0.0± 0.0	0.4± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.8± 0.2	0.4± 0.24	0.0± 0.0	1.6± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.2± 0.2
2BE (450µl)(G 4)	250	25± 0.54 a	3.8± 0.37 a	1.0± 0.0	2.2± 0.24 a	0.8± 0.24 a	2.4± 0.24 a	2± 0.31 a	4.4± 0.4 a	1.4± 0.24 a	14.2± 0.2 a	2.8± 0.2 a	4.2± 0.37 a	7.0± 0.31 a	23.2± 0.58 a
Quercein +2BE (450µl)(G 6a)	250	11.0± 0.31 abd	1.6± 0.24 abd	0.8± 0.2	1.2± 0.2a bd	0.6± 0.24 a	1.0± 0.0 ab	0.8± 0.2 b	1.6± 0.24 ab	0.8± 0.2 abd	6.8± 0.37 abd	1.2± 0.2 abd	1.4± 0.24 abd	2.6± 0.24 abd	9.6± 0.24 abd
2BE (900µl)(G 5)	250	36.4± 0.5 a	6.8± 0.37 a	1.2± 0.2	2.6± 0.24 a	1.4± 0.24 a	3.6± 0.24	3.4± 0.24 a	5.4± 0.4 a	1.8± 0.2 a	19.4± 0.24 a	4.4± 0.24 a	5.8± 0.37 a	10.2± 0.37 a	33.8± 0.58 a
Quercein +2BE(900 µl)(G7a)	250	17.0± 0.31 acd	2.8± 0.37 acd	0.8± 0.2	1.4± 0.24 acd	0.6± 0.24 ac	1.8± 0.37 ac	1.6± 0.24 ac	2.0± 0.31acd	1.0± 0.31 acd	9.2± 0.37 acd	1.6± 0.24 acd	3.0± 0.31 acd	4.6± 0.24 acd	15.4± 0.4 acd

G: group, Chd.g.: chromatid gap, Chd.br: chromatid break,Chs.g:chromosome gap, D.:deletion F.:fragment, C.A.:centromeric attenuation, Ring:ring chromosome

Table (3): Chromosomal aberration frequencies in bone marrow cells of mice treated with quercetin for 14 days with different doses of 2-butoxyethanol.

Groups	No. of scored cells	Abnormal cells (%)	Cells with more than one aberration (%)	Chromatid type aberrations (%)		Chromosome type aberrations (%)					Total structural aberrations (%)	Numerical aberrations (%)		Total num. aberr. (%)	Total aberration Excluding g. (%)
				Chd. g.	Chd .br	Chs. g	D	F	CA	Ring		Polyploidy	Aneuploidy		
Control (G1b)	250	1.4± 0.24	0.0± 0.0	0.4± 0.2	0.4 0.24	0.0± 0.0	0.0± 0.0	0.6± 0.24	0.0± 0.0	0.0± 0.0	1.4± 0.24	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.6± 0.24
Oil (G2b)	250	2.2± 0.2	0.0± 0.0	0.6± 0.2	0.4± 0.24	0.0± 0.0	0.0± 0.0	0.8± 0.2	0.0± 0.0	0.0± 0.0	1.8± 0.37	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.0± 0.0
Quercein (G3b)	250	2.2± 0.2	0.0± 0.0	0.4± 0.24	0.0± 0.0	0.6± 0.24	0.0± 0.0	0.8± 0.2	0.4± 0.24	0.0± 0.0	2.2± 0.2	0.0± 0.0	0.0± 0.0	0.0± 0.0	1.2± 0.2
2BE (450µl)(G4)	250	25± 0.54 a	3.8± 0.37 a	1.0± 0.0	2.2± 0.24 a	0.8± 0.24 a	2.4± 0.24 a	2± 0.31 a	4.4± 0.4 a	1.4± 0.24 a	14.2± 0.2 a	2.8± 0.2 a	4.2± 0.37 a	7.0± 0.31 a	23.2± 0.58 a
Quercein +2BE (450µl)(G6b)	250	4.8± 0.37	0.0± 0.0	0.4± 0.24	0.6± 0.24 abd	0.4± 0.24	0.8± 0.2 ab	0.6± 0.24 b	1.0± 0.0 ab	0.0± 0.0	3.8± 0.37 abd	0.4± 0.24 bd	0.6± 0.24 bd	1.0± 0.0 abd	4.0± 0.31 abd
2BE (900µl)(G5)	250	36.4± 0.5 a	6.8± 0.37 a	1.2± 0.2	2.6± 0.24 a	1.4± 0.24 a	3.6± 0.24	3.4± 0.24 a	5.4± 0.4 a	1.8± 0.2 a	19.4± 0.24 a	4.4± 0.24 a	5.8± 0.37 a	10.2± 0.37 a	33.8± 0.58 a
Quercein +2BE (900µl)(G7b)	250	8.2± 0.37	0.0± 0.0	0.6± 0.24	1.0± 0.0ac	0.8± 0.2 c	1.4± 0.24 ac	1.0± 0.0 c	1.2± 0.2 acd	0.0± 0.0	6.0± 0.31 acd	1.0± 0.0 acd	1.2± 0.2 acd	2.2± 0.2 acd	6.8± 0.2 acd

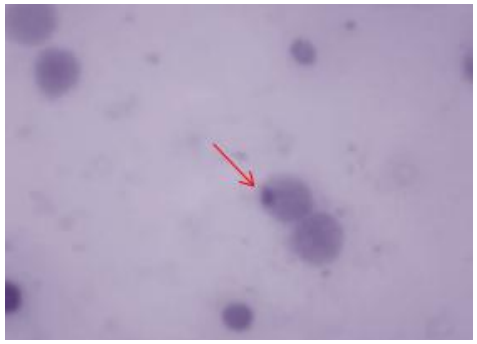

Fig. (1): Photomicrograph showing micronucleus (at 400X magnification) induced at higher percentage by the treatment of mice by 2-butoxyethanol at two different doses. Quercetin (antioxidant) was found to reduce this percentage and its effect was better at 14 days than that of 7 days.

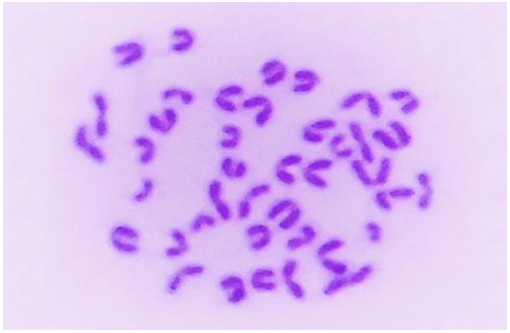

Fig. (2): Photomicrograph of normal metaphase spread from bone marrow cells (1000 X magnifications) of mice in the control group showing normal chromosomes.

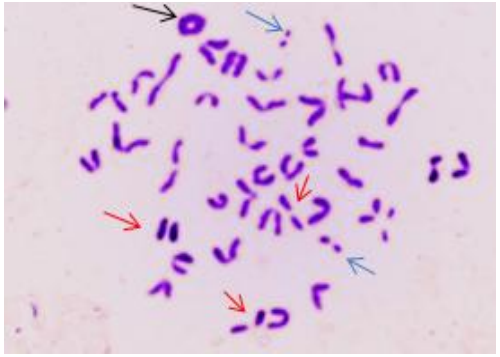

Fig. (3): Photomicrograph of metaphase spreads prepared from the bone marrow cells (1000 X magnification) of mice orally treated with 2-butoxyethanol at different doses induced: ring chromosome (black arrow) , fragments (blue arrows) & centromeric attenuation (red arrow). Quercetin was found to reduce this percentage and its effect was better at 14 days than that of 7 days.

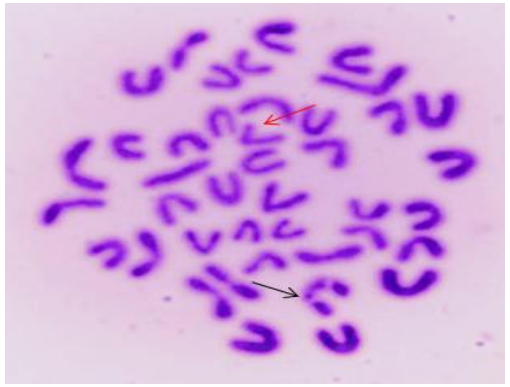

Fig. (4): Photomicrograph of metaphase spreads prepared from the bone marrow cells (1000 X magnifications) of mice orally treated with 2BE at different doses. It induced: gap (black arrow) & deletion (red arrow) in chromosomes. Quercetin is found to reduce the percentage of chromosomal aberration and abnormal cells as it is a powerful antioxidant, it is able to scavenger reactive oxygen species (free radicals) formed by 2-butoxyethanol in the cells , these free radicals damage DNA and hence cause defects in the chromosomes