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Effects of Quercetin on Acute Toxicity of Rat Spleen and Chromosome Aberrations in Bone Marrow Induced by Nickel Chloride

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Abstract

Quercetin, one of the major flavonoids in some fruits and vegetables, has much stronger antioxidative and anticarcinogenic activities. The aim of this study was to evaluate the protective role of quercetin in attenuating the histological changes of spleen and chromosome aberrations in rat bone marrow after $NiCl_2$ acute poisoning. Male Wistar rats received either $NiCl_2$ alone in the dose of 50, 150, and 300 mg/kg and pretreatment with quercetin in the dose of 50, 100, and 200 mg/kg for one hour before administration of $NiCl_2$ 300 mg/kg by single oral gavage. Spleen was removed and cells in bone marrow were collected at 24 hours after treatments. The results showed that the histology of spleen was observed to remain unchanged following exposure to all doses of $NiCl_2$. However, the chromosome aberrations were increased significantly by $NiCl_2$ 300 mg/kg as compared to the control group ($p < 0.001$). Interestingly, the increase of chromosome aberrations induced by $NiCl_2$ 300 mg/kg could be reduced significantly by quercetin pretreatment at dose of 100 and 200 mg/kg ($p < 0.05$), but not in 50 mg/kg group. Our findings provide evidence that quercetin at dose of 100 and 200 mg/kg had a possible effect to protect against $NiCl_2$ induced chromosome aberrations in rat bone marrow. The possible mechanisms of quercetin may be involved its property in act as a free radical scavenger, or its indirect action in reducing the level of oxygen reactive species.

Key words : quercetin, chromosome aberrations, spleen toxicity, nickel chloride

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1. Introduction

Nickel (Ni) is a useful heavy metal. It is used extensively in electroplating, in the manufacture of steel and other alloys and in the manufacture of batteries and electronic devices. Many Ni compounds are released into the atmosphere during mining, smelting, and refining operations. Ni is a widely used chemical in the preparation of a number of industrial and household-based products, but inhalation or ingestion of nickel (II) induces multiple toxic effects in both human and animals (Roberts et al, 1992; Zhicheng, 1994). At the same time, the high consumption of Ni-containing products in modern times inevitably leads to more exposure of humans to Ni and its by-products at all stages of production, recycling and disposal. Ni and its compounds have been reported to be potent carcinogens and toxic agents in humans and experimental animals. Therefore, Ni compounds are considered to be an industrial/occupational health hazard (IARC, 1990).

Ni compounds are well-established human carcinogens. However, the precise molecular mechanism of Ni carcinogenesis is undefined. It is known that many transition metals can damage DNA by facilitating production of free radicals generated by various mechanisms including Fenton chemistry (Sarkar, 1995). Ni is a redox active metal and indirect damage due to generation of reactive oxygen species (ROS) is probably important in Ni toxicity and carcinogenesis (Kasprzak, 1991).

Ni compounds have been shown to produce single-strand breaks in cellular DNA, as well as chromosomal aberrations and DNA-protein crosslinks (Patierno and Costa, 1985; Misra et al, 1993). It has been suggested that Ni was not directly involved in the DNA-protein crosslinks but was catalyzing it through indirect mechanisms, which may include the formation of oxygen radicals (Kasprzak, 1991; Lin et al, 1992). Exposure to nickel, either by inhalation or ingestion, has multiple effects on the immune system, including thymic involution and decreased spleen T cell numbers (Dieter et al, 1988; Haley et al, 1990; Smialowicz et al, 1984).

Some natural antioxidant products have been shown to protect cells from oxidative injury. Flavonoids are best known for their antioxidant properties, and may act in vitro as reducing agents, hydrogen donors, free radical quenchers and metal ion chelators (Shahidi, 1992). Quercetin (3,5,7, 3′,4′-pentahydroxy-flavone) is one of the most commonly occurring flavonoids and is ingested in edible fruits and vegetables at levels of up to 16 mg per day (Hertog et al, 1993). It has been shown to possess anticarcinogenic abilities, which are attributable to its anti-oxidative capacity (van Acker et al, 1996) or to other mechanisms of anticarcinogenicity in animal studies (Stavric, 1994). The study noted above suggest a potentially effect of quercetin and provide insight as to how the antioxidant effects occur in vitro. Evidence of efficacy in vivo is more limited.

As there are no reports on the acute in vivo genotoxic effect of $NiCl_2$ in rat bone marrow by the oral route, the present study was undertaken to obtain an insight into the acute effects of oral administration of $NiCl_2$ on chromosome aberrations. In addition, spleen is one of the principle sites for the initiation of most primary immune responses, for B lymphocyte activation and the production of antibodies. There is no information regarding the possible role of quercetin on the histological changes of spleen. Therefore, we planned experiments to investigate whether the quercetin at dose of 50, 100 and 200 mg/kg could protect against the acute toxicity of $NiCl_2$ on histological changes of spleen and chromosome aberrations in bone marrow.

2. Materials and Methods

2.1 Chemicals

Nickel chloride ($NiCl_2$) (prepared in distilled water), quercetin dihydrate (suspend quercetin

dihydrate in 0.5% carboxy methylcellulose in distilled water) and colchicine were purchased from Sigma-Aldrich, USA. The other chemicals were purchased from the following suppliers; KCl, methanol, absolute ethanol, acetic acid from Merck chemicals (Darmstadt, Germany).

2.2 Animals and treatment

Adult male Wistar rats weighing 150-180 g were used in this study. The experimental animals were supplied by the National Laboratory Animal Center of Mahidol University and used for experiments after 1 week of acclimatization. The animals were maintained on a standard feed with drinking water ad libitum under controlled environmental conditions. No mortality was observed throughout the experiments.

The experiment was divided into two parts. The first part was done to test the acute toxicity of $NiCl_2$ at doses of 50, 150 and 300 mg/kg on histopathological changes of spleen. The rats were separated randomly into 4 groups of 8 animals each. The first group was used as control and administered with distilled water. Groups 2-4 were administered $NiCl_2$ at dose of 50, 150 and 300 mg/kg by a single oral gavage. The second part was done to investigate the effects of quercetin on histopathological changes of spleen and chromosome aberrations induced by $NiCl_2$ 300 mg/kg. The rats were separated randomly into 5 groups of 8 animals each. Group 5 was used as control. Group 6 was administered $NiCl_2$ at dose of 300 mg/kg and group 7-9 were administered quercetin at dose of 50, 100, and 200 mg/kg for one h before administration of $NiCl_2$ 300 mg/kg by single oral gavage. The rats were killed 24 h after each treatment by anesthesia with ether.

2.3 Chromosome analysis

The animals were injected with 0.025% colchicines at dose of 0.01 ml/gm intraperitoneally before killing the animals for 2 hours to block the cells in metaphase. The cells in bone marrow were flushed by pushing 2-3 times of 0.075 M KCl into the marrow cavity of femur and tibia. Cells were collected, left in KCl for 7-8 min. and centrifuged at 1,000 rpm for 8 min. The supernatant was removed; the pellet was resuspended and fixed in 5 ml of methanol: acetic acid (3:1) for 20 min. Repeat the centrifuge and fixed cells in two or three times. Finally, the cells were dropped on a clean wet slide and uniformly stained by 5% Giemsa stain.

The classification of aberrations was carried out as described by Venitt and Parry (Venitt and Parry, 1984) and in the International System for Cytogenetic Nomenclature (ISCN) (Cohen, 1993). From each slide 100 cells were scored under 1000x magnification to determine the frequencies of chromosome damaged cells. Aberrations were pooled as described by De Flora et al, 1990. This is because deletions can only be unequivocally distinguished from achromatic lesions if the distal acentric fragment is displaced. Thus, pooling aberrations avoids artificial discrepancies between scorers due to different perceptions of the width of an achromatic lesion relative to the width of its chromatid. Accordingly, chromatid deletions and achromatic lesions were pooled as chromatid lesions. One hundred metaphases per data point were analyzed in each experiment.

2.4 Histopathological examination of spleen

Spleen was immediately removed and washing in saline solution. The spleen was fixed in 10% phosphate buffered formalin. Following an overnight fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (58-60°C). Tissue blocks were made and sectioned of 5 µm thickness with microtome. The tissue sections were stained with hematoxylin and eosin and observed under the light microscope.

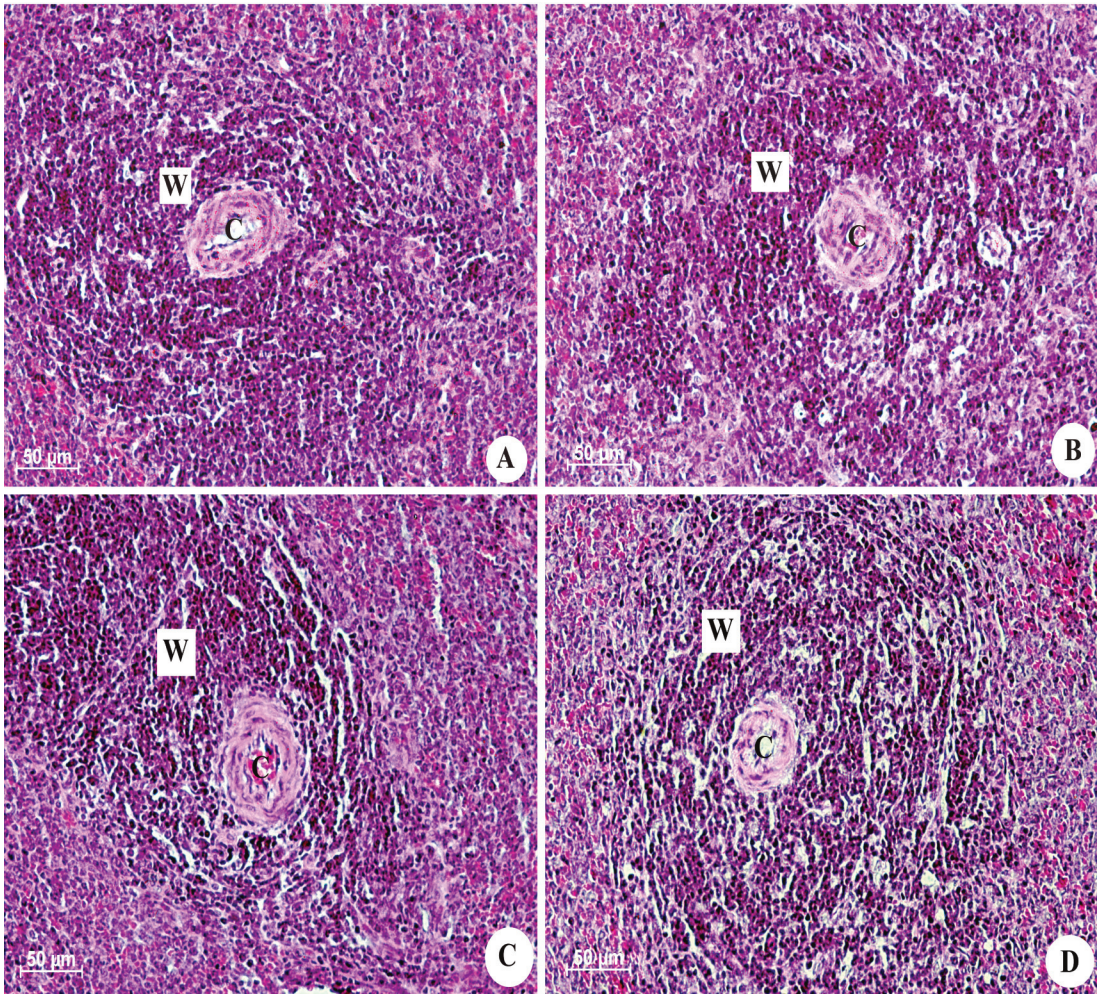

Figure 1 Representative histological microphotographs of spleen by light microscope with H&E staining at 200X magnification. The morphology of cells in spleen was not changed in all groups of treatment. (A) control, (B) $NiCl_2$ 50 mg/kg, (C) $NiCl_2$ 150 mg/kg, (D) $NiCl_2$ 300 mg/kg. These microphotographs show central artery (C) and white pulp of spleen (W).

2.5 Statistical Analysis

The SPSS for Window 13.0 computer program was used for statistical analysis. Statistical comparison of chromosome aberrations from control, $NiCl_2$ exposure and quercetin pretreatment were carried out by one-way analysis of variance (ANOVA) test. The levels of statistical significance employed in all groups were $p<0.05$ and $p<0.001$. Results were expressed as means ± S.E.M.

3. Results

3.1 Acute toxicity of $NiCl_2$ on histopathological changes of spleen

Histological studies of spleen from various treatment groups are represented in figure 1 and 2. The histology of spleen was observed to remain unchanged following exposure to $NiCl_2$ at dose of 50, 150, 300 mg/kg. The morphology of splenocytes in all groups of treatment was not different from control group (Figure 1).

3.2 Effects of quercetin on histopathological changes of spleen induced by $NiCl_2$ 300 mg/kg

The histology of spleen was observed to remain unchanged following pretreatment with

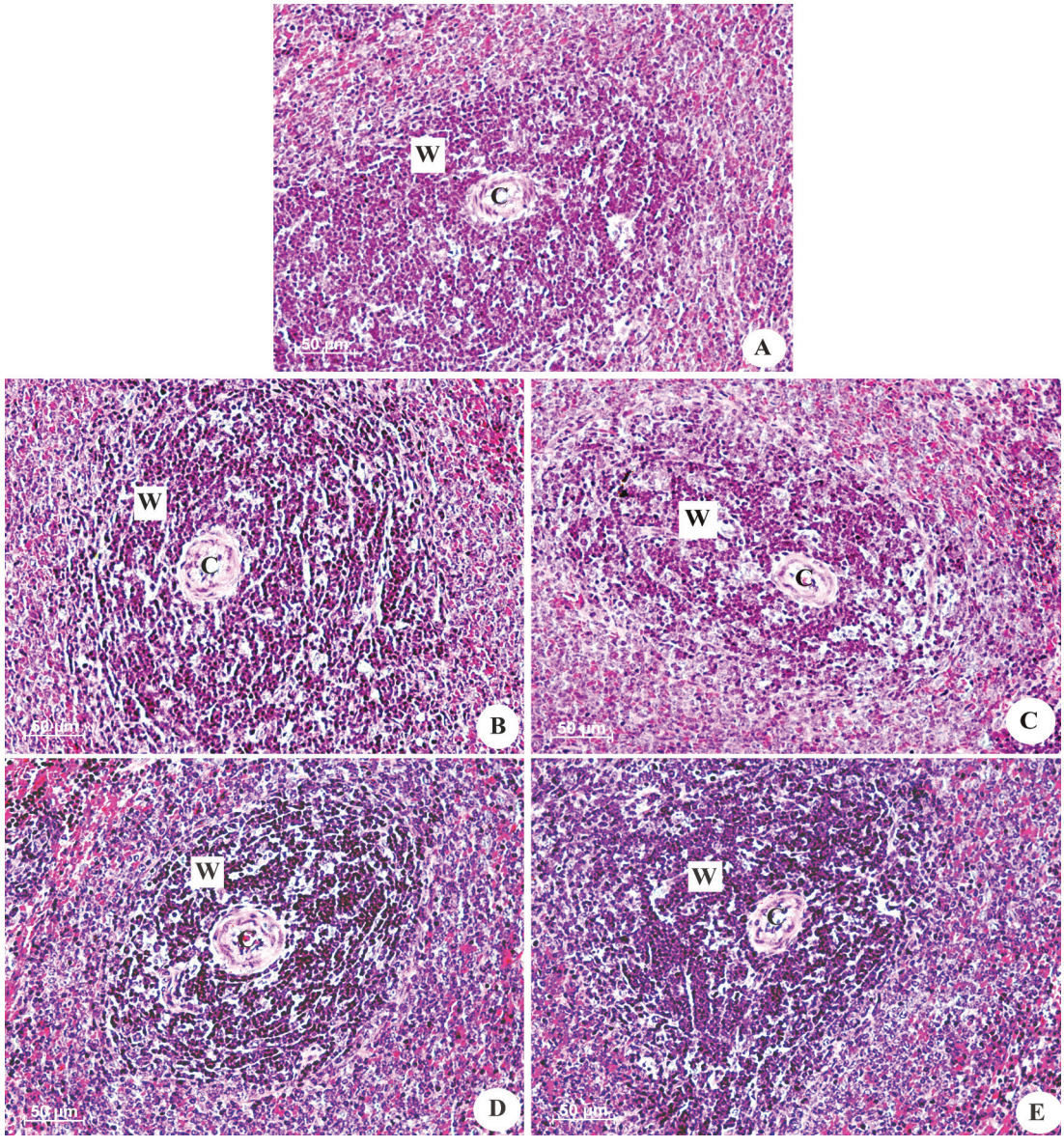

Figure 2 Representative histological microphotographs of spleen by light microscope with H&E staining at 200X magnification. The morphology of cells in spleen was not changed in all groups of treatment. (A) control, (B) $NiCl_2$ 300 mg/kg, (C) quercetin 50 mg/kg and $NiCl_2$ 300 mg/kg, (D) quercetin 100 mg/kg and $NiCl_2$ 300 mg/kg, (E) quercetin 300 mg/kg and $NiCl_2$ 300 mg/kg. These microphotographs showed central artery (C) and white pulp of spleen (W).

quercetin at dose of 50, 100 and 200 mg/kg before administration of $NiCl_2$ 300 mg/kg. (Figure 2).

3.3 Effects of quercetin on chromosome aberrations induced by $NiCl_2$ 300 mg/kg

The percentage of chromosome aberrations was increased by $NiCl_2$ 300 mg/kg as compared to the control values (p<0.001). Interestingly, the increased percentage of chromosome aberrations induced by $NiCl_2$ 300 mg/kg could be reduced significantly by quercetin pretreatment at dose of 100 and 200 mg/kg (p<0.05), but not in 50 mg/kg group. The greatest reduction of chromosome aberrations was obtained in the quercetin 100 mg/

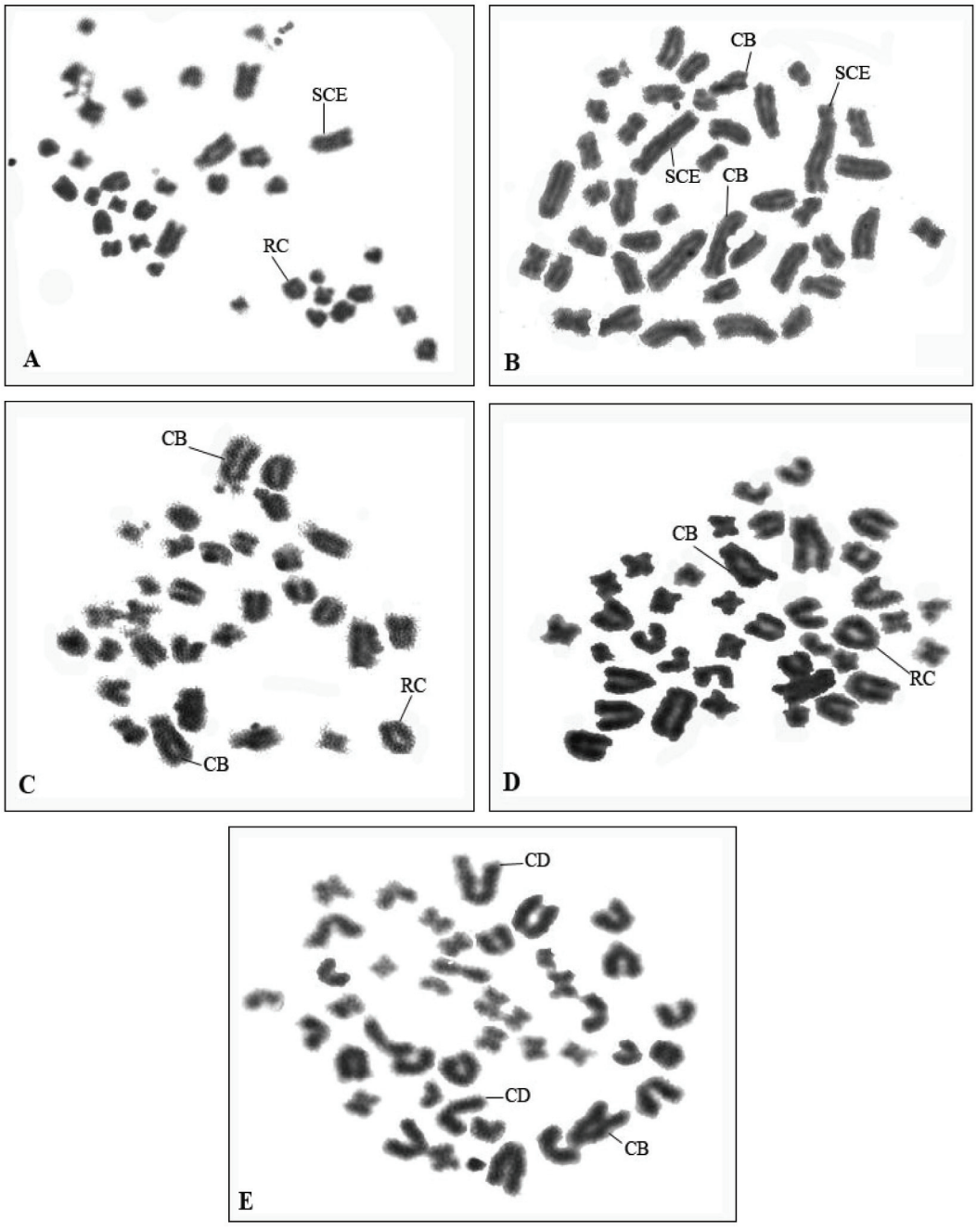

Figure 3 Effects of $NiCl_2$ and quercetin on chromosome aberrations. Each photograph was representative of the entire chromosome aberrations from rat bone marrow and had 1000X magnification. This photograph showed the different types of chromosome aberrations that found in this experiment A) sister chromatid exchange (SCE) and ring chromosome (RC) from the control group, B) chromatid breaks (CB) and SCE from $NiCl_2$ 300 mg/kg group, C) CB and RC from quercetin 50 mg/kg and $NiCl_2$ 300 mg/kg group, D) CB and RC from quercetin 100 mg/kg and $NiCl_2$ 300 mg/kg group, E) chromatid deletion (CD) and CB from quercetin 200 mg/kg and $NiCl_2$ 300 mg/kg.

Table 1 Observed percentage of chromosome damage in rat bone marrow after a single acute oral doses of $NiCl_2$ 300 mg/kg and pretreatment with quercetin 50, 100 and 200 mg/kg before administration with $NiCl_2$ 300 mg/kg.

Groups of treatment	% chromosome aberrations
Control	15.00 ± 1.77
$NiCl_2$ 300 mg/kg	33.25 ± 2.25**[a]
Quercetin 50 mg/kg + $NiCl_2$ 300 mg/kg	35.25 ± 2.49**[a]
Quercetin 100 mg/kg + $NiCl_2$ 300 mg/kg	22.00 ± 0.81*[ab]
Quercetin 200 mg/kg + $NiCl_2$ 300 mg/kg	26.75 ± 1.70*[ab]

Data were expressed as means ± S.E.M.

**[a] Significantly different from control group (p<0.001)

*[ab] Significantly different from control and $NiCl_2$ 300 mg/kg groups (p<0.05)

kg treated group (Table 1, Figure 3).

The chromosome damages occurred in this experiment had different types such as sister chromatid exchange (SCE), chromatid deletion (CD), ring chromosome (RC), chromatid breaks (CB) as shown in the Figure 3. Sister chromatid exchange and chromatid breaks were more frequently found in the $NiCl_2$ 300 mg/kg when compared to the other groups.

4. Discussion

Humans are frequently exposed to metals due to their ubiquity, widely used in industries and persistence in the environment. Ni compounds have been found to be carcinogens to humans and/or animals. Hence, the health effects of Ni have been a matter of concern because of the potential human exposure consequent to its widely spread use. Our results confirm the genetic damages induced by $NiCl_2$ exposure. The significant DNA damage observed after $NiCl_2$ treatment agrees with the results obtained from other metals like lead, chromium and cadmium (Danadevi et al, 2000, 2001; Valverde et al, 2000) in mice and mercury in rats (Grover et al, 2001) with comet assay.

Experimental evidence indicates that oxidative mechanism may be operative in the carcinogenesis and genetic toxicity of Ni compounds (Kasprzak, 1991). Ni (II) may induce genotoxic effects, such as DNA-protein crosslinking, DNA strand breaks, sister chromatid exchange, and oxidative DNA base damage (Kasprzak, 1995). One hypothesis is that Ni can produce •OH radical within the cell via a Fenton/Haber-Weiss reaction (Torreilles and Guerin, 1990) and the •OH radical could result in damaged bases (Dally and Hartwig, 1997), DNA strand breakage (Stinson et al, 1992) and DNA protein crosslinks (Misra et al, 1993, Lei et al, 1995). The genotoxic potential may also contribute to the carcinogenicity of this metal (Oller et al, 1997). The genotoxicity of $NiCl_2$ is characterized by damage to DNA repair, DNA damage by various insults may lead to cell transformation or death, but only if not repaired.

In nuclear chromatin, the DNA molecule, having an abundance of phosphate anions and nitrogen and oxygen donor groups, is an ideal binding partner for metal cations, including Ni^{2+}. The chromatin proteins can bind Ni^{2+} even stronger (Bal et al, 1998). This helps to explain why, following *in*

vivo exposure, heavy metals, including nickel, are found in cell nuclei (Berg, 1986). The generation of O_2 and H_2O_2, was also detected in cell nuclei (Peskin and Shlyahova, 1986). Hence, the bound metal can catalyze ROS generation in the cell nucleus and thus facilitate oxidative damage to DNA and other nuclear components, as observed experimentally. Important targets for metals are also mitochondria and mitochondrial DNA. The major oxidative effects in DNA associated with exposure of experimental animals and cultured cells to nickel and other transition metals include strand scission, depurination, cross-linking, and base modifications (Landolph, 1999, Kasprzak and Buzard, 2000).

Although Ni is poorly absorbed from the gastrointestinal tract, exposure via food and drinking water provide most of the intake of Ni and Ni compounds (Coogan et al, 1989). A review by the EPA (1986) revealed that humans and animals absorb approximately 1-10% of dietary Ni. Similar values were reported for drinking water exposure. Ni metal is poorly absorbed dermally but some Ni compounds such as $NiCl_2$ or nickel sulphate can penetrate occluded skin resulting in up to 77% absorption within 24 h. Ni is excreted in the urine and feces, because it is poorly absorbed, most ingested nickel is excreted in the feces (ATSDR, 1988).

The average daily intake of Ni in food is approximately 0.002 mg Ni/kg/day and the tolerable intake (TI) of $NiCl_2$ is 0.0013 mg Ni/kg/day (Haber et al, 2000). As 80-90% of nickel chloride is excreted and only small amount is retained, we had to give rats higher single doses to reflect the human exposure limits. The human exposure differs in different occupational settings. The doses we used might reflect some occupational situations. We used $NiCl_2$ at dose of 300 mg/kg to evaluate the protective effects of quercetin on histopathological changes of spleen because it was found that $NiCl_2$ 300 mg/kg could induce hepatotoxicity but not in $NiCl_2$ 50 and 150 mg/kg from our previously report. However, our experiment showed that the histology of spleen was observed to remain unchanged in all treatments with $NiCl_2$ and quercetin. These results suggested that the spleen might be not the primary target organ for acute toxicity induced by $NiCl_2$ via the oral route. Furthermore, nickel is not very toxic when introduced by the oral route and it was thought to be due to poorer absorption by this route.

Since the intracellular reduction of nickel is necessary for nickel-induced DNA damage, the effect of quercetin pretreatment on $NiCl_2$-induced chromosome aberrations was examined. For this experiment, we chose to study quercetin because of its superior antioxidant effect among other flavonoids. Its antioxidant effect was documented in many *in vitro* and *in vivo* experimental studies (Mojzis et al, 2001). Furthermore, quercetin is known as an excellent metal chelator. Recently, it was confirmed that both anti-radical and chelating effects are involved in the protective effect of quercetin (Cheng and Breen, 2000). When quercetin was administered orally, it was poorly absorbed from the digestive tract and did not have a great influence on the organs (Murota and Terao, 2003).

Quercetin is the major flavonoid in the human diet and its daily intake with foods is estimated to be 50-500 mg (Deschner et al, 1991). The protective effect of quercetin may also be accounted for, at least in part, by their ability to enhance the activity of a variety of detoxification enzymes and /or to shift the metabolic profile of carcinogens such that the intraconcentration of the reactive metabolites is diminished. The precise mechanism of the protective action of quercetin on nickel-mediated cytotoxicity is assumed to be due to the scavenging of superoxide anions that produce hydroxyl radicals via the Haber-Weiss reaction from H_2O_2 generated in this system, or by the chelation of metal ions that are used to produce highly toxic

hydroxyl radicals from H_2O_2 via the Fenton reaction. This assumption is based on the fact that hydroxyl radicals, which are constantly produced by glucose oxidase from glucose substrate, are known to act as direct sources of cellular damage (Rollet-Labelle et al, 1998).

From the present study, it can be concluded that acute $NiCl_2$ treatment had no effects on the morphological changes of spleen but it could induce chromosome aberrations in bone marrow at dose of 300 mg/kg. Furthermore, pretreatment with quercetin at dose of 100 and 200 mg/kg, but not 50 mg/kg has a possible effect to protect against the acute toxicity of $NiCl_2$ on chromosome aberrations in bone marrow. This may be caused by its antioxidant and scavenger properties.

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บทคัดย่อ

ผลของเควอซีทินต่อความเป็นพิษแบบเฉียบพลันในม้าม และความผิดปรกติของโครโมโซมในไขกระดูกของหนูขาวที่ถูกเหนี่ยวนำโดยนิกเกิลคลอไรด์

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[๔]สาขาเภสัชวิทยาและพิษวิทยา สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยวลัยลักษณ์ นครศรีธรรมราช

เควอซีทินเป็นสารฟลาโวนอยด์ที่พบในผลไม้และผัก มีฤทธิ์ในการยับยั้งการเกิดออกซิเดชัน และการเกิดมะเร็งได้ งานวิจัยนี้จึงทำขึ้นเพื่อศึกษาผลของเควอซีทินในการลดความเป็นพิษแบบเฉียบพลันต่อผลการเปลี่ยนแปลงทางพยาธิวิทยาของม้าม และความผิดปกติของโครโมโซมในไขกระดูกของหนูขาวที่ถูกเหนี่ยวนำโดยนิกเกิลคลอไรด์ หนูขาวเพศผู้สายพันธุ์วิสต้า ได้รับนิกเกิลคลอไรด์เพียงอย่างเดียวในปริมาณ ๕๐, ๑๕๐ และ ๓๐๐ มิลลิกรัมต่อกิโลกรัม และได้รับเควอซีทินในปริมาณ ๕๐, ๑๐๐ และ ๒๐๐ มิลลิกรัมต่อกิโลกรัมเป็นเวลาหนึ่งชั่วโมงก่อนได้รับนิกเกิลคลอไรด์ ๓๐๐ มิลลิกรัมต่อกิโลกรัม โดยการป้อนทางปาก ผลการทดลองพบว่านิกเกิลคลอไรด์ทั้ง ๓ ขนาดไม่ทำให้เกิดการเปลี่ยนแปลงทางเนื้อเยื่อวิทยาของม้าม แต่นิกเกิลคลอไรด์ในปริมาณ ๓๐๐ มิลลิกรัมต่อกิโลกรัมทำให้ความผิดปรกติของโครโมโซมเพิ่มมากขึ้นอย่างมีนัยสำคัญ เมื่อเทียบกับกลุ่มควบคุม ($p < ๐.๐๐๑$) ความผิดปรกติของโครโมโซมที่เพิ่มขึ้นจากการให้นิกเกิลคลอไรด์ปริมาณ ๓๐๐ มิลลิกรัมต่อกิโลกรัมนี้ สามารถลดลงได้อย่างมีนัยสำคัญ ($p < ๐.๐๕$) โดยการให้เควอซีทินปริมาณ ๑๐๐ และ ๒๐๐ มิลลิกรัมต่อกิโลกรัม แต่ไม่สามารถลดลงเมื่อให้เควอซีทินปริมาณ ๕๐ มิลลิกรัมต่อกิโลกรัม การทดลองนี้จึงสนับสนุนว่า มีความเป็นไปได้ที่จะใช้เควอซีทินปริมาณ ๑๐๐ และ ๒๐๐ มิลลิกรัมต่อกิโลกรัมในการต้านพิษของนิกเกิลคลอไรด์ที่เหนี่ยวนำให้เกิดความผิดปรกติของโครโมโซมในไขกระดูกของหนูขาว ซึ่งกลไกการป้องกันของเควอซีทินอาจมาจากฤทธิ์ในการเป็นตัวกำจัดสารอนุมูลอิสระโดยตรง หรือมาจากการลดระดับของสารอนุมูลอิสระโดยทางอ้อม

คำสำคัญ : เควอซีทิน ความผิดปรกติของโครโมโซม ความเป็นพิษต่อม้าม นิกเกิลคลอไรด์