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ORIGINAL ARTICLE

Cellular Protection Induced by Genistein in Mouse and its Antioxidant Capacity

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ABSTRACT

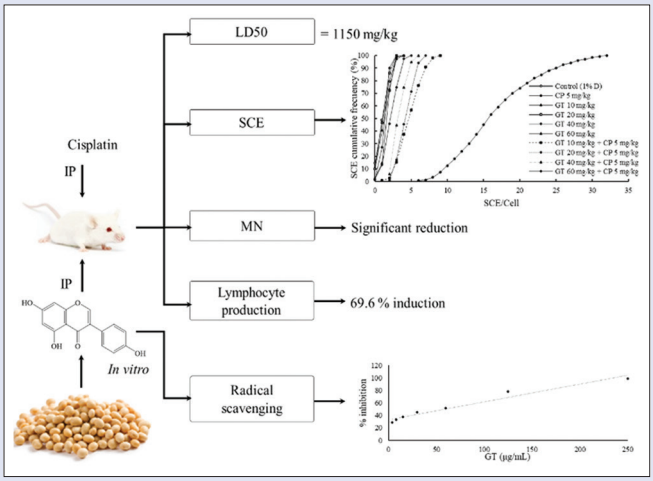
Background: Genistein (GT) is an isoflavone phytoestrogen present in a number of plants. The chemical has been reported to have antioxidant, antigenotoxic, and cancer-preventive qualities; however, no studies against cisplatin (CP) have been reported. **Objective:** The main objective of the study is to determine the capacity of GT to inhibit the genotoxic and cytotoxic damage induced by CP in mouse, as well as its immunostimulant ability and its capacity to scavenge free radicals. **Materials and Methods:** We determined the effect of six doses of GT on the rate of sister chromatid exchanges (SCEs) and of micronuclei (MN) in mice administered with 5 mg/kg of CP. Besides, we determined its capacity to increase the amount of lymphocytes in mouse and to reduce oxidation with the 2,2-diphenyl-1-picrylhydrazyl assay. **Results:** Our results showed that GT (10–60 mg/kg) significantly decreased the frequency of SCE and of MN in mice. Furthermore, we also observed a moderate bone marrow cytotoxic correction of the damage induced by CP, as shown by an improvement in the rate of polychromatic erythrocytes. In addition, with 60 mg/kg, GT increased 69.6% the production of mouse lymphocytes over the control value throughout a 72-h trial. Moreover, the compound also showed a high capacity to trap free radicals (95.25%, with 250 μg/ml). **Conclusion:** Our results, therefore, established that GT is an effective cellular protective agent against the action of CP.

Key words: 2,2-diphenyl-1-picrylhydrazyl, cisplatin, genistein, lymphocytes, micronuclei, sister chromatid exchange

SUMMARY

- Genistein (GT) reduced the number of sister chromatid exchange in mouse bone marrow treated with cisplatin (CP)
- GT remarkable reduced the frequency of micronuclei in mouse blood treated with CP
- GT was an excellent lymphocyte inductor in mouse and an *in vitro* antioxidant agent.

Abbreviations used: SCE: Sister chromatid exchanges; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DL_{50}: Dose lethal 50; MN: Micronuclei; IP: Intraperitoneal; GT: Genistein; CP: Cisplatin; LM8: Osteosarcoma cell line; PC3: Prostate cell line; LNCaP: Lymph node carcinoma of the prostate cell line; UAAC-3199: Breast cell line; OVCAR-3: Ovarian cell; ESR1: Estrogen receptor-1; ROS: Reactive oxygen species; HepG2: Human liver cancer cell; NOX1: NOX1 gene; Nrf2: Nrf2 transcription factor; SOD1: Superoxide dismutase 1; HO-1: Heme oxygenase-1; MNPE: Micronucleated polychromatic erythrocytes; DMBA: 7, 12-dimethylbenz (A) anthracene; HGPRT: Hypoxanthine-guanine phosphoribosyltransferase receptor; V79: V79 cell line; HONE6: HONE6 cell line; BrdU: 5-bromodeoxyuridine; KCl: Potassium chloride; CPK: Cellular proliferation kinetics; M1, M2, M3: Mitosis 1, 2, 3; AGT: Average generation time; MI: Mitotic index; PBS: Phosphate-buffered saline; PE: Polychromatic erythrocytes; NE: Normochromic erythrocytes; SDM: Standard deviation mean; OD: Optical density; Fe^{2+}-ADP: Fe^{2+}-ADP complex; H_2O_2: Hydrogen peroxide.



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INTRODUCTION

Isoflavones are phytoestrogen compounds chemically similar to 17-β-estradiol hormone whose consumption provides several beneficial effects for human health.[1] Genistein (GT) [Figure 1] is a compound that belongs to this group. It is found in different plants with nutritional properties such as in the red clover and in soybeans. In fact, the aforementioned plant is recognized to constitute the most abundant source of isoflavones.[2] Soybeans, in addition to GT, also contain aglycones (daidzein and glycitein) and their respective acetyl, malonyl, and glucoside conjugated forms.[3,4]

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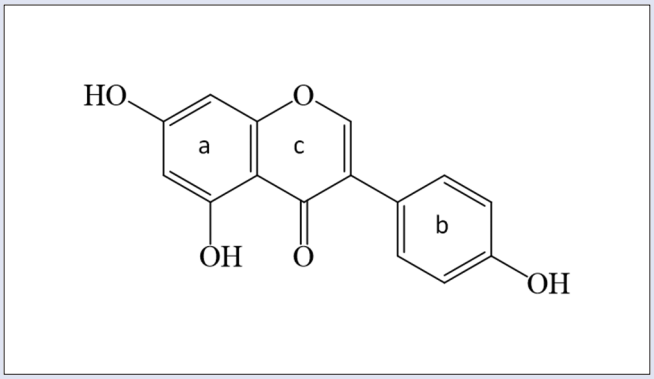

Figure 1: Chemical structure of genistein. The figure shows two aromatic rings (a and b) linked by a heterocyclic pyrane ring (c)

GT has received remarkable interest regarding its potential effect on the prevention of cancer. Several studies have demonstrated its ability to inhibit the activities of the enzymes tyrosine kinase and topoisomerase II, effects that have been related with antiproliferation, cell transformation inhibition, or with proapoptotic properties attributed to the compound.[5,6]

Studies on various tumor cultivated cell lines, including osteosarcoma, prostate (lymph node carcinoma of the prostate cell line), breast, and ovarian cells, have confirmed the anticarcinogenic capacity of GT. Such studies have reported induction of differentiation,[7] inhibition of angiogenesis,[8] antiproliferation related with the hypermetilation of the gene BRCA1 in the estrogen receptor-1,[9] as well as a proapoptotic effect by increasing the expression of the P14 tumor suppressor gene.[10]

Moreover, GT has been reported to decrease the reactive oxygen species (ROS) level, which, for example, has been evaluated in the human liver cancer cell line, where it was attributed to the suppression of the translation and activation of NOX1, the disruption of the mitochondrial electron transport chain system, as well as to the scavenging of ROS through the Nrf2 transcription factor-mediated induction of Phase II antioxidant enzymes, such as superoxide dismutase 1 (SOD1) and heme oxygenase-1.[11]

Besides, reports have also shown the resistance of various tumors to cisplatin (CP) treatment, as well as the improvement of its cytotoxic efficacy when the mentioned antineoplastic is combined with GT. This effect has been mainly observed in cellular lines, including nonsmall cell lung cancer, Henrietta Lacks (HeLa) cervical cancer, human medulloblastoma, hepatocellular carcinoma, and pancreatic cells.[12-16] The mentioned authors have observed an additive or synergistic effect between the two compounds and have speculated that the elevation of tumor cell mortality by CP may be related with the nuclear factor-Kb and Akt/mammalian target of rapamycin pathways inhibition by GT, metabolic routes that are known to downregulate the activity of various antiapoptotic genes.

CP is a chemotherapeutic agent which in its hydrolyzed form binds the N7 atom on purine residues and may cause DNA damage, blocks cell division, and produces apoptotic cell death.[17] Moreover, the mutagenicity of the agent has been evaluated in various biological systems with positive effects, which include the DNA damage by measuring mutations at the hypoxanthine-guanine phosphoribosyltransferase receptor locus in hamster V79 cells,[18] the induction of lethal recessive genes identified in the wing spot test in *Drosophila melanogaster*,[19,20] the sister chromatid exchange (SCE) increase after 72 h of exposure in HONE6 cells,[21] and the induction of micronuclei (MN) in nontarget cells of mice.[22]

Furthermore, it has been well documented that cancer development is intimately connected with the induction of DNA and chromosome damage, and therefore, that blocking or retarding such effects by antigenotoxic agents may reduce cancer incidence. In this field, the use of GT has been reported to decrease both the DNA and chromosome damage induced by various mutagens. Authors have determined the reduction of DNA strand breakage induced by peroxyl free radicals in plasmid DNA strands pBR322 isolated from *E. coli* RRI strain[23] the inhibition of micronucleated polychromatic erythrocytes (MNPE) induced by 7,12-dimethylbenz(A) anthracene (DMBA) in bone marrow of Wistar rats,[24] or the number of chromosome aberrations and SCE induced by norethandolone and oxaldrolone in human peripheral blood cultures.[25] However, few reports have been published concerning the specific GT antitoxic or antigenotoxic effect over the CP damaging potential, a fact that sustains the relevance of the present research. A significant protection of the compound was determined on various parameters that were elevated in CP-induced renal injuries in mice; in this study, the administration of GT decreased ROS production, the expression of monocyte chemoattractant protein-1, the intercellular adhesion molecule 1, the level of apoptosis, and the infiltration of macrophages.[26] In another report on an investigation made in cultured human lymphocytes, the authors observed a significant correction by GT on the number of chromosomal aberrations, SCE, and cell cycle kinetics which were drastically altered by the action of CP.[27] Moreover, in a pretreatment of GT given to cultured human lymphocytes damaged with CP,[28] it was observed a reduction in the expression of caspase 3, a protein that cleaves and activates caspases 6 and 7, which play a central role in the execution of cell apoptosis. Therefore, based on the previous observations, which refer to the GT capacity to decrease ROS, its anticarcinogenic potential and the few *in vitro* reports that show its protection toward the CP genotoxic effects, the general aim of the present investigation was to further contribute to the evaluation of the protective capacity of GT. For this purpose, we analyzed the GT antigenotoxic effect over the damage induced by CP in two mice *in vivo* assays; in one of them, we measured its inhibitory effect on the number of MNPE and in the other, we examined its inhibitory effect on the number of SCE. Besides, its antioxidant property was measured with the 1,1-diphenyl-2-pycril-hydrazyl (DPPH) assay, as well as its lymphocyte stimulating effect in mouse blood.

MATERIALS AND METHODS

Chemicals and animals

GT 98% pure was obtained from Sigma-Aldrich (Mexico City), whereas 5-bromodeoxyuridine (BrdU), CP, dimethyl sulfoxide, colchicine, and DPPH were purchased from Sigma Chemicals Co., (St. Louis, MO, USA). The Giemsa and the Wright stains were obtained from Merck (Mexico City). Sodium citrate, sodium chloride, potassium phosphate, and sodium phosphate were purchased from Baker S. A. (Mexico City).

CD1 male mice, every individual weighing 25 ± 2 g, were obtained from Harlan Laboratories (Mexico City). They were kept in metal cages with six animals each, at 23°C ± 1°C and a 12-h dark–light cycle (8 am–8 pm). Animals consumed food (Rodent Laboratory chow 5001, Purina) and water *ad libitum*. The experimental protocol was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the National Rehabilitation Institute.

Lethal dose 50 of genistein

We determined the lethal dose 50 (LD_{50}) using a method consisting of two steps and using thirteen animals for the whole assay.[29] Mice were intraperitoneally (IP) administered with the chemical and mortality was observed up to 14 days. The LD_{50} was obtained as the geometric mean

between the minimal LD and the maximal sublethal dose. With this procedure, we confirmed the previously reported LD_{50} of 1150 mg/kg for GT.[30] Surviving animals were sedated with sodium pentobarbital (40 mg/kg) and cervically dislocated by a trained co-author.[31] In the next two cytogenetic assays (SCE and MNPE), the same procedure was followed.

Determination of sister chromatid exchanges, cellular proliferation kinetics, and mitotic index

For this assay, ten experimental groups with six mice each were IP injected with the compounds and organized as follows: a negative control group administered with 0.4 ml of 1% dimethyl sulfoxide[32] which was the vehicle of GT; a positive control group treated with 5 mg/kg of CP; four groups injected with 10, 20, 40, and 60 mg/kg of GT; and finally, four other groups treated first with the same amounts of GT and 30 min later with 5 mg/kg of CP. The selected dose of the mutagen was based on previous studies that have shown significant genotoxic effects without systemic toxicity.[33]

A 50 mg tablet of BrdU partially coated with paraffin (60% of its surface) was subcutaneously implanted in each animal, and 1 h later, the experimental mice were injected with the chemicals. Twenty-one hour after the tablet implantation, mice were IP injected with 5 mg/kg of colchicine and left for 3 h; then, the animals were cervically dislocated, their femurs dissected and the obtained bone marrow was placed in a solution of KCl (0.075 M) at 37°C and incubated for 30 min. The cell suspension was centrifuged for 10 min at 625 × g, the supernatant was discarded and the cells were fixed in a solution of methanol-acetic acid (3:1). The fixation process was repeated at least twice. Finally, for the staining process, we deposited three drops of each cell suspension onto ethanol-cleaned slides and treated them to differentiate the sister chromatid according to the method described earlier.[34,35]

The cytogenetic analysis per mouse was made as follows: (1) the rate of SCE was determined in 60 second-division metaphases; (2) the cellular proliferation kinetics was determined in 100 metaphases, identifying the cells in first (M1), second (M2), and third (M3) cellular division. With these data, we determined the average generation time (AGT) using the formula AGT = (22/[M1] + [2][M2] + [3][M3]) × 100; and (3) the Mitotic Index (MI) was determined in 1000 cells. A statistical test of the obtained data was initially made with a one-way ANOVA followed by a two-tailed Student's *t*-test, using the program Instat 2 (GraphPad Software, Inc. San Diego, CA, USA).

Micronucleated polychromatic erythrocytes

Ten groups with six individuals each were IP inoculated with the tested compounds and organized as follows: a negative control group was treated with 1% dimethyl sulfoxide, another group was administered CP (5 mg/kg), the following four groups were injected with GT (10, 20, 40, and 60 mg/kg), and finally, the last four groups were first treated with GT (10, 20, 40, or 60 mg/kg), and 30 min later, they were administered with 5 mg/kg of CP.

To carry out the micronucleus examination, before the chemical administration, we obtained two drops of blood from the tail of each mouse and smeared them onto ethanol-cleaned slides; then, the cells were fixed in methanol for 3 min, stained for 15 min with a 4% Giemsa solution made in phosphate-buffered saline (pH 6.8) and gently rinsed in running water.[33] Subsequently, the tested chemicals were administered to mice and the blood cells were obtained and stained as indicated above at 24-, 48-, 72-, and 96-h postadministration.

To determine the antigenotoxic potential of GT, we scored the rate of MNPE in 2000 polychromatic erythrocytes (PE) per mouse, and for the anticytotoxic effect, we identified the rate of PE and normochromic erythrocytes in 2000 erythrocytes per mouse.[36]

For the statistical analysis of the obtained data, we initially applied a two-way ANOVA for repeated measures, which was followed by a two-tailed Student's *t*-test, using the program Instat 2.

Free radical scavenging potential

This study was based on the measurement of substances that scavenge the stable radical DPPH. Following the method described by Russo *et al.*,[37] we prepared an 86 μM solution of DPPH in ethanol, and afterward, we added 4, 8, 15, 30, 60, 125, and 250 μg/ml of GT. The absorbance of the mixture was measured at λ = 517 nm, 10 min after the addition of the tested chemical. The experiment was made in triplicate and the percentage of the scavenging activity was calculated using the formula ([control absorbance − GT absorbance]/control absorbance) × 100. Results were evaluated with a linear regression analysis.

Lymphocyte counting

Six groups of mice with five individuals each were IP injected with the tested compounds. Four of these groups were administered with GT (10, 20, 40, and 60 mg/kg), another group with 0.4 ml of 1% dimethyl sulfoxide and the last group was injected with the immunostimulant agent α-interferon (0.01 μl/kg), which is a dose that falls within the therapeutic range reported for rat and human beings and one that has been applied to increase the lymphocyte production in mouse.[38] Twenty-four hour after the chemical administration, we obtained two drops of blood from the tail of each mouse and smeared them on ethanol-cleaned slides; the cells were fixed in methanol and stained with Wright solution for 5 min, the color was accentuated with distilled water for 6 min, and finally, the slides were rinsed in tap water. We then made a differential count of white blood cells including neutrophils, eosinophils, basophils, lymphocytes, and monocytes. A statistical test of the obtained data was made with a one-way ANOVA, followed by a two-tailed Student's *t*-test using the program Instat 2.

RESULTS

Sister chromatid exchange, average generation time, and mitotic index

With respect to the rate of SCE, mice treated with CP revealed an increase of about eight times the level observed in the negative control group [Table 1]. The same table also shows that the four groups treated with different doses of GT had homogeneous values without significant differences with respect to the negative control. On the contrary, the SCE values in animals treated with the four doses of GT before being administered with CP showed a significant decrease with respect to the CP treated mice, with a mean inhibition value of 78.5%; however, such inhibition did not reach the control level, having values significantly higher in all cases. Table 1 also shows that the range of most AGT values went from 12 to 13 h and that no specific modification in such parameter was induced by the tested agents. The results for MI presented no modifications among the experimental groups.

Figure 2 shows the cumulative frequencies of the determined SCE numbers in all experimental groups. Such parameter revealed a constant increase of the cumulative SCE frequencies in the CP-treated mice, low frequencies in the control and the GT-treated animals and certain SCE cumulative displacement in the combined groups, which was inversely related with the increase of the applied GT doses. In fact, an SCE dose-dependent effect with respect to GT administration was detected. For example, animals administered 10 mg/kg of GT plus 5 mg/kg of CP had a high displacement, indicating lower protection against the damage

Table 1: Sister chromatid exchanges, average generation time, and mitotic index in mice treated with genistein and cisplatin

Agent	Dose (mg/kg)	SCE±SDM	M1 (%)	M2 (%)	M3 (%)	AGT (h)	MI (%) ± SDM
Control	1% D	2.51±0.12[b]	35	50	15	12.22	5.94±0.22
CP	5	18.06±0.12[a]	38	50	12	12.64	6.14±0.19
GT	10	2.36±0.16[b]	35	52	13	12.35	6.01±0.16
GT	20	2.18±0.22[b]	35	51	14	12.29	5.92±0.16
GT	40	2.21±0.40[b]	36	53	11	12.57	6.12±0.13
GT	60	2.20±0.26[b]	33	51	16	12.02	5.96±0.13
GT+CP	10+5	4.56±0.16[a,b]	34	52	14	12.22	6.11±0.16
GT+CP	20+5	4.31±0.14[a,b]	36	53	11	12.57	6.02±0.18
GT+CP	40+5	3.51±0.54[a,b]	37	52	11	12.64	6.32±0.16
GT+CP	60+5	3.14±0.28[b]	35	53	12	12.42	5.86±0.23

[a]Statistically significant difference with respect to the control value and; [b]With respect to CP values. All determinations were made in six mice per group. One-way ANOVA and *post hoc* two-tailed Student's *t*-test ($P \leq 0.05$). SCE was determined in 60 second division bone morrow cells. Each value represents the mean±SDM. M1, M2, M3: Percentage of cells in first, second, and third cellular division scored in 100 cell per mouse. AGT was determined in 100 cells per mouse with the formula AGT: (22/[M1] + [2][M2] + [3][M3]) × 100. M) was scored in 1000 cells per mouse. D: Dimethyl sulfoxide (0.4 mL/mouse); SCE: Sister chromatid exchange; SDM: Standard deviation mean; AGT: Average generation time; MI: Mitotic index; CP: Cisplatin; GT: Genistein

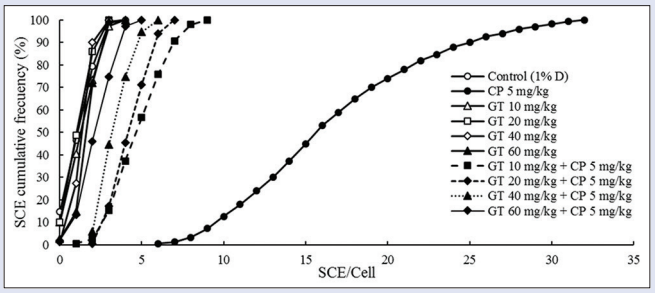

Figure 2: Sister chromatid exchanges cumulative frequencies in mice treated with cisplatin and genistein. D = 1% dimethyl sulfoxide (0.4 mL/mouse). Each line portrays the cumulative frequency of sister chromatid exchanges displayed in 60 second-division metaphases per group ($n = 6$)

generated by the mutagen; however, with the increase of the GT doses, a gradual decrease in the displacement curve was observed, approaching the control group curve, which indicates a gradual higher reduction in the DNA damage.

Results regarding the determination of MNPE are shown in Table 2. CP increased the number of MN since the first observation time (24 h), with an average value of 24.7 MNPE/cell in the 4-day assay, a value that was highly significant with respect to the control level. Animals treated only with GT induced certain MN increase; however, with respect to the effect of CP, the MNPE decrease exerted by GT was evident with a mean of 84.29% in the whole evaluated time. In the experiment, the high dose of GT (60 mg/kg) showed the best protective effect.

Results regarding cytotoxic effects are shown in Table 3. In this case, a significant PE number decrease induced by CP was found at all examined time points. Such decrease almost reached four times the level observed in the control group and indicated a strong inhibition of bone marrow cell proliferation; however, by adding GT before the mutagen a complete correction of the damage was observed. Besides, no bone marrow cell proliferation damage by the tested isoflavone was observed.

Free radical scavenging potential

With the DPPH assay, we demonstrated a strong capacity of GT to trap free radicals [Figure 3]. The mean ± standard deviation mean of the optical density (OD) obtained for the tested concentrations of GT (μg/mL) were as follows: $OD_{GT4} = 0.225 \pm 0.002$; $OD_{GT8} = 0.212 \pm 0.003$; $OD_{GT15} = 0.197 \pm 0.006$; $OD_{GT30} = 0.172 \pm 0.002$; $OD_{GT60} = 0.139 \pm 0.001$; $OD_{GT125} = 0.066 \pm 0.003$; $OD_{GT250} = 0.003 \pm 0.002$; $OD_{control} = 0.329 \pm 0.002$. The effect was expressed in a concentration-dependent manner reaching a DPPH radical inhibition as high as 99% with 250 μg/ml of GT. The data

Table 2: Micronucleated polychromatic erythrocytes in mice administered genistein and cisplatin

Agent	Dose (mg/kg)	Percentage MNPE, ± SDM			
		24 h	48 h	72 h	96 h
Control	1% D	1.7±0.16[b]	1.8±0.22[b]	1.8±0.19[b]	1.65±0.32[b]
CP	5	22.1±0.26[a]	23.7±0.22[a]	22.3±0.21[a]	24.72±0.22[a]
GT	10	4.16±1.22[a,b]	4.92±0.46[a,b]	3.86±0.16[a,b]	3.22±0.19[a,b]
GT	20	3.66±1.32[a,b]	4.44±0.48[a,b]	4.32±0.18[a,b]	3.46±0.12[a,b]
GT	40	3.64±1.14[a,b]	3.44±1.24[a,b]	2.48±0.14[a,b]	2.66±0.32[a,b]
GT	60	2.42±0.87[a,b]	1.78±0.42[a,b]	2.26±0.12[a]	2.32±0.62[a,b]
GT+CP	10+5	6.6±0.26[a,b]	6.9±0.22[a,b]	6.1±0.18[a,b]	5.4±0.19[a,b]
GT+CP	20+5	6.2±0.28[a,b]	6.1±0.06[a,b]	5.1±0.24[a,b]	5.1±0.44[a,b]
GT+CP	40+5	4.3±0.18[a,b]	3.8±0.21[a,b]	3.3±0.38[a,b]	4.1±0.22[a,b]
GT+CP	60+5	2.4±0.22[b]	2.0±0.26[b]	1.7±0.64[b]	2.1±0.12[a,b]

[a]Statistically significant difference with respect to the control value and [b]with respect to CP values. One-way ANOVA and *post hoc* two-tailed Student's *t*-test ($P \leq 0.05$). Each value represents the mean±SDM obtained in 1000 polychromatic erythrocytes per mouse. Six mice per group. D: Dimethyl sulfoxide (0.4 mL/mouse); MNPE: Micronucleated polychromatic erythrocyte; SDM: Standard deviation mean; CP: Cisplatin; GT: Genistein

Table 3: Effect of genistein and cisplatin on mouse bone marrow cytotoxicity

Agent	Dose (mg/kg)	Percentage PE, ± SDM			
		24 h	48 h	72 h	96 h
Control	1% D	1.8±0.18[b]	2.1±0.21[b]	2.0±0.42[b]	1.9±0.36[b]
CP	5	0.4±0.44[a]	0.3±0.18[a]	0.5±0.22[a]	0.6±0.06[a]
GT	10	1.9±0.24[b]	2.2±0.26[b]	1.8±0.41[b]	2.3±0.32[b]
GT	20	2.1±0.12[b]	2.2±0.18[b]	2.4±0.21[b]	1.8±0.26[b]
GT	40	1.8±0.38[b]	1.9±0.18[b]	2.1±0.12[b]	2.3±0.35[b]
GT	60	2.2±0.35[b]	2.0±0.12[b]	1.9±0.31[b]	1.9±0.48[b]
GT+CP	10+5	1.2±0.24[a]	1.4±0.20[a,b]	1.4±0.26[a,b]	1.4±0.22[a,b]
GT+CP	20+5	1.9±0.12[b]	1.2±0.16[a,b]	1.4±0.23[a,b]	1.8±0.28[b]
GT+CP	40+5	1.6±0.38[b]	1.5±0.11[a,b]	1.8±0.11[b]	1.9±0.15[b]
GT+CP	60+5	1.5±0.35[b]	1.5±0.18[a,b]	1.7±0.44[b]	1.6±0.38[b]

[a]Statistically significant difference with respect to the control value and [b]with respect to CP values. One-way ANOVA and *post hoc* two-tailed Student's *t*-test ($P \leq 0.05$). % PE was registered with respect to the percentage of normochromic erythrocytes. Each value represents the mean±SDM obtained in 1000 erythrocytes per mouse. Six mice per group. D: Dimethyl sulfoxide (0.4 mL/mouse); PE: Polychromatic erythrocyte; SDM: Standard deviation mean; CP: Cisplatin; GT: Genistein

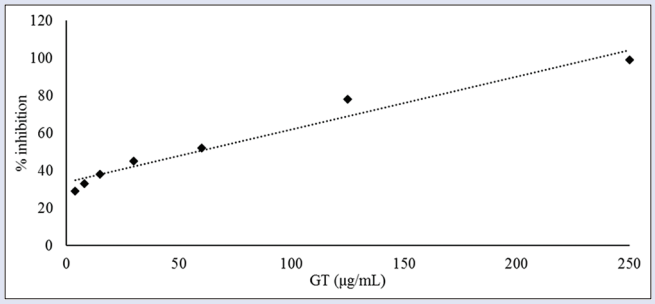

Figure 3: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity induced by genistein. Absorbance at 517 nm was scored after 10 min of exposure at room temperature. The percentage 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was calculated according to the equation: % 2,2-diphenyl-1-picrylhydrazyl radical scavenging = ([Control absorbance – GT absorbance]/[Control absorbance]) (100). $y = 1.02x + 37$. $r = 0.94$

corresponded to a linear tendency ($y = 1.02x + 37$) with a correlation coefficient $r = 0.94$ ($P = 0.0005$).

Lymphocyte counting

In regard to the induction of lymphocytes by GT, Table 4 shows a significant increase of these cells with the four tested doses at all evaluated times. The highest induction was determined with the administration of 60 mg/kg of GT (74.5%). The other types of blood cells in GT-treated animals were found in a range similar to those determined for the control animals.

DISCUSSION

Our present results regarding MNPE and SCE showed a significant protective effect of GT on the DNA damage induced by CP when the tested antigenotoxic agent was preadministered. Our results were not dose dependent suggesting that the tested dose range was appropriate to show the effect. This could be in line with the absence of MI decrease by GT showed in Table 2 and with the homogeneous PE numbers observed with all doses of the compound [Table 3]. Furthermore, our study clearly demonstrated the absence of DNA damage potential by the studied isoflavone. Its observed protective effect is obviously related with the CP mechanism(s) of action. In this field, one of its actions is the formation of DNA adducts through its binding with the N7 atom of purine residues, which in this form cause DNA damage and block cell division.[17] Regarding this type of effect by CP, the studied isoflavone has been reported to be able to inhibit DMBA initiation/promotion skin tumorigenesis, precisely by blocking DNA adduct formation.[39] Besides this activity, DNA double-strand breaks induced by CP in replicating but not in quiescent cells have been reported as the cause of DNA repair inhibition and the promotion of various cellular damage effects.[40] In this aspect, GT has been observed to substantially decrease the plasmid DNA breaking level induced by peroxyl free radicals,[23] and in this respect, evidence has suggested that peroxyl radical initiates strand damage on the complementary strand via C4'-hydrogen atom abstraction, producing double-strand breaks.[41] In fact, there is substantial information suggesting that the subjacent explanation for the genotoxic and cytotoxic potential of CP is connected with its generation of oxidative stress, which has been observed in both normal and malignant cells.[42,43] Accordingly, there is also well-sustained evidence about the GT antioxidant capacity, including data of the present report.

In this context, GT has been reported to effectively quench free radicals produced by toxic agents and to protect cells against oxidative damage

Table 4: Lymphocyte induction in mice administered with genistein

Agent	Dose (mg/kg)	Percentage lymphocyte, ±SDM			
		24 h	48 h	72 h	96 h
Control	1% D	42.5±0.12[b]	41.8±0.26[b]	44.1±0.16[b]	42.4±0.08[b]
α-interferon	0.01 µl/kg	51.3±0.22[a]	56.2±0.32[a]	60.4±0.12[a]	59.0±0.18[a]
GT	10 mg/kg	56.6±0.21[a,b]	60.1±0.18[a,b]	62.5±0.18[a]	67.6±0.42[a,b]
GT	20 mg/kg	61.8±0.22[a,b]	61.3±0.28[a,b]	64.8±0.22[a,b]	66.4±0.21[a,b]
GT	40 mg/kg	62.1±0.14[a,b]	64.6±0.16[a,b]	70.3±0.52[a,b]	71.7±0.24[a,b]
GT	60 mg/kg	66.4±0.16[a,b]	67.1±0.16[a,b]	74.5±0.22[a,b]	73.8±0.38[a,b]

[a]Statistically significant difference with respect to the control value and [b]with respect to α-interferon values. One-way ANOVA and *post hoc* two-tailed student's *t*-test ($P \leq 0.05$). Each value represents the mean±SDM per group. Six mice per group. D: Dimethyl sulfoxide (0.4 mL/mouse); SDM: Standard deviation mean; GT: Genistein

at DNA level by inhibiting microsomal lipid peroxidation induced by Fe^{2+}-ADP complex, or to induce antioxidant enzymes such as SOD and catalase;[44,45] also, a pretreatment with GT to human lymphocytes previously damaged with hydrogen peroxide showed a significant reduction in the DNA strand breaks measured with the comet assay;[46] in another study, a marked inhibition by GT was demonstrated on the number of DNA strand breaks induced by hydrogen peroxide/Cu(II) and hydroquinone/Cu(II) in ΦX-174 plasmid DNA;[47] besides, the supplementation of GT to Jurkat T-cell line and to peripheral blood lymphocytes of healthy individuals treated with hydrogen peroxide showed a significant DNA and oxidative damage protection, evaluated with the comet and the lipid peroxidation assays.[48] Furthermore, the marked geno/cytotoxicity induced by the chemotherapeutic agent bleomycin is known to be related with the generation of oxygen radicals, which can produce oxidative cleavage over DNA strands and have apoptotic potential.[49] Regarding these effects, a pretreatment with GT has been observed to decrease the MN frequency and the DNA damage determined by single-cell gel electrophoresis assay in human lymphocytes.[50] Therefore, the provided information about GT clearly suggests two points regarding its antigenotoxic potential: initially, that the compound may act in various ways to protect cellular damage, and therefore, it can be a valuable antigenotoxic agent; and second, that its strong antioxidant capacity explains a vast part of its protective activity.

Another interesting property of GT is its capacity as immunomodulatory agent. Various reports have sustained such effect with respect to viral damage, autoimmune encephalomyelitis, mouse cervical cancer, or mouse hematopoietic damage by ionizing radiation injury.[51-54] In the mentioned health problems, similarly to that observed in the present report, the authors have found lymphocyte proliferation induced by GT as well as induction of specific immunocompetent cells.

CONCLUSION

Our present report established a strong antigenotoxic potential of GT against cisplastine in mouse, as well as a significant lymphocyte and antioxidant induction; moreover, it also suggested that the molecule could be valuable to prevent the DNA damage because of its versatile ways of acting on the genetic material. However, specific studies concerning its mechanism(s) of action against mutagens with various types of effects are highly advisable.

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Conflicts of interest

There are no conflicts of interest.

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