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Research article

Evaluation of cellular safety and the chemical composition of the peanut (*Arachis hypogaea* L.) ethanolic extracts

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ABSTRACT

Arachis hypogaea L. (Leguminosae) is distributed in tropical and subtropical areas. Peanut has high nutritional and commercial value. Scientific research showed that peanut has biological properties such as anticancer, antioxidant, antiinflammatory. However, it is necessary to know if consumption of peanut, either as food or as a phytopharmaceutical implies a health risk. The aim was to evaluate cytotoxicity and genotoxicity of ethanolic extracts from *A. hypogaea*. Also, chemical characterization of these extracts was performed. Cytotoxicity was evaluated by MTT and Neutral Red Uptake (NRU) assays on Vero cells. Genotoxicity was studied by Micronuclei and comet assays on Balb/C mice. Qualitative and quantitative chemical analysis of extracts were performed. Results showed that extracts have low cytotoxicity. Tegument ethanolic extract (TEE) and Seed ethanolic extract (SEE) were not genotoxic. The treatments with TEE at 250 mg/kg and SEE at 2000 mg/kg revealed (highest concentrations evaluated) some toxicity on blood marrow cells of mice. Chemical characterization indicated that TEE had 74.33 ± 1.10 mg GAE/g of dried extract and SEE had 15.05 ± 0.06 mg GAE/g of dried extract of total phenolic content. Also, proanthocyanidins (O.D. at 550 nm 1.39 ± 0.15) and caffeic acid (2.46%) were identified in TEE. While, linoleic acid (58.84%) oleic acid (11.31%) and palmitic acid (8.37%) were major compounds of SEE. In conclusion, peanut consumption is safe at concentrations recommended for healthy uses, such as nutrition, and phytomedicine.

1. Introduction

The genus *Arachis* is native from South America and includes 80 species. *Arachis hypogaea* L. stands out as the most economically important species (Lopes et al., 2011). It is a member of the family Leguminosae, which is distributed in tropical and subtropical areas (El-Sayed et al., 2012). It is also cultivated in Asia, Africa, and America, mainly as raw material to produce human food and vegetable oil (Lopes et al., 2011).

China is one of the largest peanut producers in the world with around 20% of the planted area and more than 40% of production (Yin et al., 2017). Argentina is also one of the main competitors at international level, due to high exportable volumes and recognized commercial quality of the product. Argentinean peanut production is concentrated in the center-south of Cordoba, where it reaches approximately 94% of national production and 3% of world production. Among the manufactures that are elaborated for human consumption, we can find: confectionary

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peanut, prepared and flavored peanut, peanut paste, peanut butter, peanut oil and peanut flour (Benencia and Fernandez, 2017).

Peanut has high nutritional and commercial value because it is an excellent source of energy (564 kcal/100 g) and nutrients, such as proteins, fatty acids, carbohydrates, dietary fibre, calcium, iron, magnesium, phosphorous, zinc, copper, and thiamin (King et al., 2008; Sebei et al., 2013; Liu et al., 2017). Moreover, it has bioactive compounds such as phenolic acids, flavonoids, phytosterols, and tocopherols (Kris-Etherton et al., 2008). Scientific research reported that consumption of 28–42 g/day is associated with health benefits (Jones et al., 2014). Flavonoids and stilbenoids, which have been identified as biologically active compounds in peanuts, were reported to have anticancer, anti-nitric oxide production, and antibacterial activity (El-Sayed et al., 2012). Resveratrol is a stilbenoid and phytoalexins, which has potent antioxidant activity. Stilbenoids also showed immunological activity (Jiang et al., 2014). Phytosterols, especially β-sitosterol, possess antitumor effects in colon, prostate, and breast (Lopes et al., 2011). Furthermore, it was found that vitamin E and polyphenols may regulate glucose metabolism and inflammation (Barbour et al., 2015). Thus, these bioactive compounds can be used as phytopharmaceuticals. Besides being broadly consumed by people for food purpose, peanut is also used in the manufacture of cosmetics, pharmaceuticals, and surfactants (Sebei et al., 2013). Studies have shown that peanut oil has hydrating effects in human skin and it protects the skin from UV radiation (Zhai et al., 2003; Korac and Khambholja, 2011).

Pharmacological activity of medicinal herbs is associated with presence of different chemical compounds that can produce a beneficial effect on the body or can be toxic. Therefore, in development of a new drug of vegetable origin it is necessary and mandatory to carry out an evaluation of its safety (García and Nora, 2003). Predictive toxicity tests that include genotoxic and cytotoxic evaluation are essential to detect if the product has ability to induce changes in DNA and if it produces adverse effects on cells or cellular mechanisms (Principales ensayos para determinar la citotoxicidad de una sustancia, 2003).

Therefore, the aim of the present study was to evaluate cytotoxicity and genotoxicity of the ethanolic extracts from *Arachis hypogaea* L. Also, the chemical characterization of these extracts were performed.

2. Materials and methods

2.1. Plant material and extraction

Peanut is an economically important legume used for direct consumption as well as for manufacturing numerous food products. Argentina is one of the major peanut producers in the world and about 90% of its crop is produced in the province of Córdoba. This crop in some way represents what this province is and the challenges it affronts to get better results over the time. To accomplish the objectives, production units in Córdoba are using the so called “Good agricultural practices” and basically, with these techniques, the province is producing health food, without the usage of any type of chemical and biological contaminants. The basis of this obtainment is to grow the crop by the mix of nutritional and chemical components inherent on the origin soil. There are some institutions that follow the process, analyzing the peanut, and responding with the collected data to the Argentine Peanut Foundation (www.camaradelmani.org.ar/english/argentine-peanut-foundation/).

Generally, in our area it is not necessary the usage of chemical fertilizers, but it is a common practice the biological fertilization throughout rhizobial inoculation with the purpose of reducing the use of agrochemicals. Rhizobia are symbiotic bacteria that elicit formation of nodules on the roots of legume, within which the bacteria fix atmospheric nitrogen into ammonia. For that, the rhizobial inoculation have been extended in the last few years, inclusive to studies of phenotypic and genotypic characteristics of native nodulating strains for selection and application and for development of successful inoculation strategies (Nievas et al., 2012). These advances also involve the use of direct rhizobial inoculation into the soil, prior to sowing, termed “in-furrow inoculation”, since it has some advantages over seed inoculation (Bogino et al., 2011), in addition by co-inoculation with selected strains of *Bradyrhizobium* and *Azospirillum* (Vicario et al., 2016).

Peanut is cultivated in Cordoba between spring, (when the soil has a temperature of 18 °C, minimum, at a depth of 10 cm for about 3 consecutive days) and autumn (april–may). The major part of authors notice that it is require approximately 500 mm of water during the cycle, well distribute in order to achieve a satisfactory peanut production (Boote and Ketring, 1990). In Córdoba, although the precipitations vary considerably comparing different years, the annual average throws a quantity of approximately 800 mm per year. Other important data is that the annual temperature average is of 18 °C, with January as the warmest month (31 - 17 °C), and July as the coldest (19 - 4 °C), as maximum and minimum temperature respectively (www.cordobaturismo.gov.ar/clima/). The soil of this part of the country has the particularity of being sandy, also this area is windswept, and all together, adding that after peanut harvest the soil is removed, contributes to the erosion of the soil. For that reason, many local producers sow winter crops to help with the preservation of the soil's nutrients.

Peanut seeds (*A. hypogaea* L. cv Manigran and cv Granoleico) were obtained from Criadero El Carmen (Gral. Cabrera, Córdoba, Argentina). Seeds harvested in 2017 were used. The extraction processes were carried out between 2017 and 2018. The harvested seeds were kept at 4 °C before the extraction processes. Briefly, the seeds and their tegument were separated and extracted with ethanol/water (80:20) for 48 h at 37 °C, according to García et al. (1990) with slight modifications. Then, the mixtures were filtered and dried at < 40 °C for 3 days. Stocks were prepared at 5 mg/ml in phosphate buffered saline (PBS), filtered with Whattman Nº 2, sterilized with 0.22 μm pore cellulose acetate filters and stored at -20 °C. Thus, seed ethanolic extract (SEE) and tegument ethanolic extract (TEE) were obtained.

2.2. Cytotoxicity assessment (in vitro)

Vero cell line was cultured in DMEM supplemented with 10% FBS, 3% L-glutamine, and 1% gentamicin solution at 37 °C with 5% CO_2. Cells were plated at 3×10^4 with 200 μl of medium per well in 96-well culture plates, and incubated overnight to achieve cellular monolayers. Then, they were treated with 100 μl of each concentration of extract (TEE and SEE) diluted in maintenance medium (with 2% of FBS) at concentrations of 5–1600 μg/ml. Assays were carried out in triplicate of four independent plates. Cellular controls (cell monolayers with medium alone) were included. The culture plate was incubated at 37 °C with 5% CO_2 for 2 days.

2.2.1. Neutral red uptake (NRU)-based assay

After incubation, cells were washed with PBS and 200 μl/well of NR solution (30 μg/ml in MEM) were added. Afterwards, plates were incubated at 37 °C for 3 h. After this time, they were washed with PBS and a mixture of acetic acid, ethanol and water (1:50:49) was added. After the agitation of cultures for 20 min, optical density values (O.D.) were measured at 540 nm using a spectrophotometer (Labsystems Multiskan MS). The relative viability was expressed as percentage of captured NR decrease with respect to control cells. A dose-response curve was constructed and analyzed statistically to determine the 50% cytotoxic concentration (CC_{50}).

2.2.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide metabolism (MTT)-based assay

The assay was made following the methodological specifications described by Mosmann (1983), using the Vybrant MTT Cell Proliferation Assay Kit (Molecular Probes Invitrogen Detection Technologies, Eugene, Oregon, USA). After removing treatments, 100 μl of medium without serum and 10 μl of MTT solution (5 mg/ml of MTT in PBS 0.01 M, pH 7.2) were added to each well. Plates were incubated for 4 h and the resulting

formazan crystals were solubilized with dimethylsulfoxide. O.D. was measured at 560 nm in a spectrophotometer (Labsystems Multiskan MS). CC_{50} determination was made in the same way that was described for the NRU test.

2.3. Genotoxicity assessment (in vivo)

Eight-week-old Balb/C mice of 25 g were used (obtained from the UNRC Central Bioterium). Mice were divided into ten groups consisting of 3 males and 3 females in each one. They were maintained in a temperature and humidity-controlled room, with a 12-h light/dark cycle, and received commercial balanced diet and water *ad libitum*. All procedures were approved by Comité de Ética de la Investigación Científica (COEDI) of Universidad Nacional de Río Cuarto (UNRC), in accordance with current legislation. Accordingly, they received by intraperitoneal injection 0.2 ml of TEE at doses of 25, 50, 100, and 250 mg/kg of body weight, or 0.2 ml of SEE at doses of 500, 1000, and 2000 mg/kg b.w. prepared in physiological solution (PS) and dimethylsulfoxide (DMSO). The negative control group received only vehicle (25 μl of DMSO in 775 μl of PS), whereas the positive control group received 20 mg/kg b.w. of cyclophosphamide. At 24 h post-injection, mice were sacrificed by decapitation without being anesthetized, and blood and femoral bone samples were immediately taken.

2.3.1. Micronuclei assay

It was done according to Schmid (1975), with slight modifications. Femurs were removed and to flush out bone marrow with FBS using a syringe, and collect it into a tube. The collected cells were centrifuged at 1000 rpm for 5 min. They were resuspended in the remaining fluid, and slides were prepared and fixed by soft flame. Then, slides were stained with May-Grünwald-Giemsa (Merck Company, Germany). The proceeding was performed for 3 min with undiluted May-Grünwald solution; then, 2 min with diluted May-Grünwald, and subsequently, in diluted Giemsa for 10 min. Then, the slides were observed using an optical microscope at a 1000 x magnification. To establish the genotoxic capacity of TEE and SEE, the micronucleated polychromatic erythrocytes (MNPCE) were scored in a total of 1000 polychromatic erythrocytes (PCE) by duplicate per mouse. To detect the possible cytotoxic effects, the PCE/NCE (normochromatic erythrocytes) ratio every 1000 PCE counted was considered.

2.3.2. Comet assay

The comet assay was carried out according to Singh et al. (1988) with slight modifications. Heparinized blood was diluted in 1 ml of FBS/RPMI (1:1) and centrifuged 5 min at 1500 rpm. The cell pellets were embedded in 75 μl of low melting point agarose at 0.75% and were extended on slides covered with a film of normal melting point agarose at 0.75%. Two slides were prepared for every concentration and the cell suspensions in agarose were allowed to gel at 4 °C. Later, 75 μl of low melting point agarose 0.75% at 37 °C were added and the slides were allowed to gel. They were submerged in a cold lysing solution (NaCl 2.5 M, EDTA 100 mM, Tris 10 mM, pH 10) and left to rest at 4 °C for 1 h. They were placed in an electrophoresis camera of higher pH (>13), exposed with alkali for 20 min (4 °C, 250 mA and 30 V) to allow DNA unwinding, submerged in neutralization buffer (Tris-HCl 0.4 M, pH 7.5) for 15 min and fixed in ethanol at 100% for 10 min. Then, they were dyed with 25 ml of ethidium bromide. The dyed cores were visualized using a fluorescence microscope (Axiophot, Carl Zeiss, Alemania) connected to an image analysis system (Powershot G6, 7.1 megapixeles, Canon INC., Japan, with the software AxioVision Release 4.6.3, Carl Zeiss, Alemania), with an excitation filter of 515–560 nm and a barrier filter of 590 nm. 100 "nucleoids" were photographed from each treatment. The highly damaged cells were not included in the score (clouds were not analyzed). Tail Moment (TM) was used to estimate DNA damage (arbitrary units).

2.4. Extract chemical characterization

2.4.1. Total phenolic content of extracts

The total phenolic content of TEE and SEE was determined by the Folin-Ciocalteu method, according to Nepote et al. (2004). Briefly, 8.4 ml of distilled water and 0.1 ml of an extract dilution (500 μg/ml) were mixed. Then, 0.5 ml of Folin-Ciocalteu reagent was added to the mixture and incubated for 6 min. Subsequently, 1 ml of saturated sodium carbonate solution was added and left for 1 h in the dark. Absorbance was measured at 760 nm. The calibration curve was with gallic acid solutions in ethanol/water (70:30), with results being expressed as gallic acid equivalents (GAE) in mg per gram of dried extract.

2.4.2. Total proanthocyanidin content of TEE

Proanthocyanidin characterization was performed according to Mabry et al. (1970); Markham (1982), Ricco et al. (2004), and Ricco et al. (2015). TEE (1 g) was extracted with 10 ml of methanol/water (80:20), for 24 h at room temperature. The methanol extract obtained was dried and dissolved in 5 ml of 2 N HCl. Later, it was incubated into a bath at 100 °C for 1 h. Control (100 μg/ml of procyanidin A2 in methanol), and standards were submitted to the same procedure. The change of red colour intensity indicated a positive reaction (transformation of proanthocyanidins into anthocyanidins). These colored compounds (anthocyanidins) can be quantified by spectrophotometer (Labsystems Multiskan MS). For this, 0.50 ml aliquots of the extract were taken and transferred to test tubes. Then 3.0 ml of the butanol-HCl reagent (butanol-HCl, 95:5 v/v) and 0.1 ml of ferric reagent (2% ferric ammonium sulfate in 2M HCl) were added. After mixing the tubes they were placed in a water bath to boil for 60 min. Finally, the absorbances were measured at 550 nm against a blank. The total proanthocyanidin content was expressed as optical density (O.D.) at 550 nm.

2.4.3. Total phytosterol content of TEE

The Lieberman-Burchard test was used (Foy Valencia et al., 2005), by extracting 0.5 g of TEE with chloroform. For then, 1 ml of acetic anhydride and 5 drops of $H_2SO_{4(C)}$ were added to 1 ml of the chloroform extract. The reaction was considered positive when a bluish green coloration appeared. The result was informed: (-) absence; (+) presence.

2.4.4. HPLC of TEE

For the qualitative and quantitative analysis of components present in the TEE sample the procedure reported by Larrauri et al. (2016) was followed. For this a Varian Pro Star chromatograph (210 model, 04171 series) was used, equipped with a UV-Vis detector and a Microsorb MV-100-5 C18 column, particle size 5 μm (250 mm length x 4.6 mm i.d., Varian). A solvent system ramp that involved 0.5% formic acid (v/v) (Solvent A) and methanol/0.5% formic acid (Solvent B) and 0.4 ml/min flow rate was used.

The injection volume of the samples was 20 μl and the detection was made at a wavelength of 290 nm. The identification was made by comparing the retention times (t_R) of the components present in the extract with t_R of controls (resveratrol, rutin, quercetin and caffeic acid, all from Sigma-Aldrich, USA).

Quantization of caffeic acid was achieved by external calibration method interpolating the area under the peak for this compound from the corresponding calibration curve of standard. The validation of the method was carried out taking into account the guidelines established by the International Harmonization Conference (ICH) and the guide for the Bioanalytical Methods Validation Industry Administration of Medicines and Foods (FDA) (The International Conference on Harmonisation of Technical Requirements, 2005; US Food and Drug Administration, 2013). Therefore, a series of assays were performed, including the determination of linearity and linear range, limits of detection (LOD) and quantification (LOQ) and intra- and inter day precision and accuracy. The equation obtained for each calibration curve and its linear correlation coefficient (r2) was: $Y = 4.583 \times 106\ X$ ($r2 = 0.99992$, $p < 0.0001$).

These data demonstrated, in the range of concentrations tested, compliance with the Lambert-Beer law and together with the results of the t-test, confirmed that the method is linear. In addition, the other validation values obtained using the method described above and that show that this HPLC method suitable for the detection and quantification of caffeic acid in the extract were: linear range 6.92–130 μg/ml; intra-day RSD 5.82%; inter-day RSD 4.12%; baseline noise ~0.0001 AU; LLOD 0.38 μg/ml; LLOQ 0.63 μg/ml; accuracy 97.1 ± 8.3%; recovery 97.5 ± 5.1%.

2.4.5. Fatty acids of SEE

Fatty acids present in the SEE were determined. The separation, quantification, and identification of total FA methyl ester resultants were performed using a capillary column (20 m length x 250 mm id x 0.25 mm film tickness, SUPELCO©, USA) of polyethylene glycol in a Clarus500© (Perkin-Elmer®) GC and all fatty acids were identified using a commercial standard (Nu-check, USA®) (Repossi et al., 2017). All values were expressed as the total percentage area (Dain et al., 2016).

2.5. Statistical analysis

All data obtained were evaluated using the *GraphPad Prism* program version 6.0 (San Diego, USA). For cytotoxicity studies, values were expressed as mean ± standard deviation (SD). Assays were carried out in triplicate of four independent plates. The data were submitted to Nonlinear regression analysis and compared with the parametric *t*-test.

For micronuclei assay, the mean ± SD of MNPCE/1000 PCE and PCE/NCE (%) of each group was calculated (six animals per group). In order to select the appropriate analyzing test, the distribution of data was evaluated by *Shapiro–Wilk* test. MNPCE/1000 PCE data were not normal, and were analyzed by the *Kruskal–Wallis* test, whereas normal PCE/NCE data were analyzed by ANOVA ($p < 0.05$). The *Kruskal Wallis* test and a posteriori Dunns multiple comparisons were used for comet assay data.

3. Results and discussion

3.1. Citotoxicity

The cytotoxicity of TEE and SEE was analyzed using MTT reduction and NRU assays. Figure 1 shows the effects of different extract concentrations on Vero cell cultures. The addition of TEE caused up to 60% cellular viability decrease after 2 days of treatment (Figure 1 A). Therefore, the CC_{50} value was more than 1600 μg/ml. Instead, the CC_{50} value of SEE was 1600 μg/ml (Figure 1 B). In short, these results indicated that both extracts had low toxicity on Vero cells evaluated by NRU assay. In contrast, the MTT assay revealed that TEE was more toxic than SEE for Vero cells, as it decreased cellular viability by 50% at 600 μg/ml, whereas SEE did not present cytotoxicity at the concentrations tested (Figure 1 C and D, respectively). When comparing the results obtained with both tests used to determine cytotoxicity, we can infer that both extracts caused more mitochondrial than lysosomal damage, as they showed lower viability values in the MTT than in the NRU assay.

There is no background regarding the study of cytotoxicity of peanut seed or tegument extracts. However, studies of cytotoxicity using ethanolic extract of *Albizia lebbeck* (legume) bark determined through the MTT technique showed alterations in HeLa cells at concentrations higher than 1000 μg/ml (Shagun et al., 2015). SEE presented a similar degree of cellular toxicity.

TEE and SEE toxicity was similar to that of the peanut leaf hydroalcoholic extract, which at concentrations of 1, 10, and 100 μg/ml neither produce cell damage after 24 h of incubation (Cossetin et al., 2019).

3.2. Genotoxicity studies

3.2.1. Micronucleus test in mouse bone marrow

The analysis of the data presented in Table 1 and Figure 2 revealed that the genotoxicity indices obtained in the animals treated independently with each concentration of the extracts, and in the control group that received DMSO vehicle, showed significant differences in relation to the positive control group but did not differ with the negative control

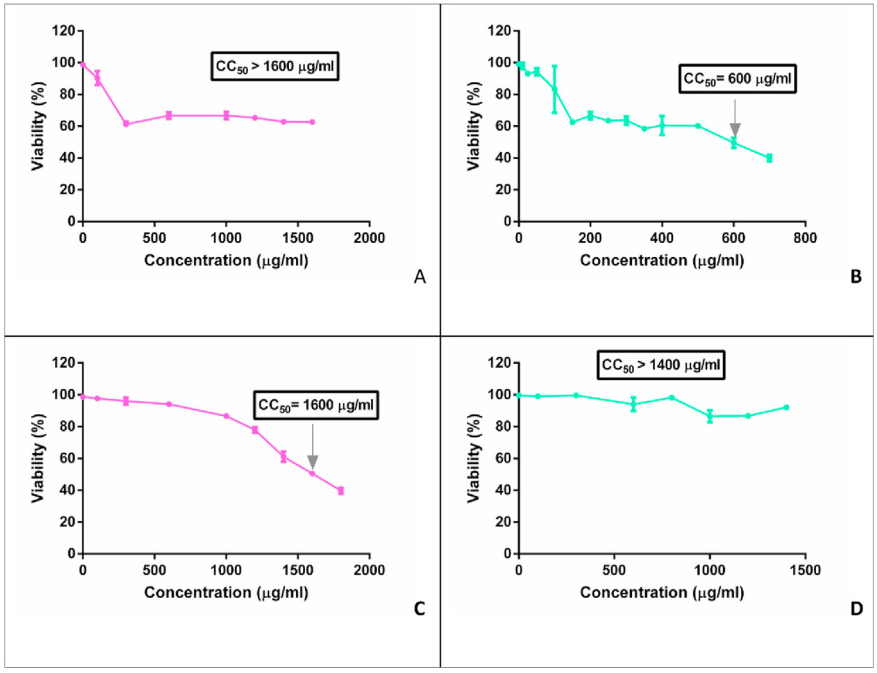

Figure 1. Curves of Vero cells viability treated with extracts, TEE (A and B) and SEE (C and D) from *Arachis hypogaea* L. Evaluations were made by NRU (A–C) and MTT reduction (B–D), then of 2 days of treatment.

Table 1. Assessment of the cytogenotoxic action in bone marrow cells of Balb/c mice treated with different concentrations of TEE and SEE from *Arachis hypogaea* L.

Treatment	Genotoxicity Index (average ± DS)	Toxicity Index (average ± DS)
Negative Control (Physiological solution)	8.5 (±1.29)	1.32 (±0.17)
Positive Control (Cyclophosphamide)	27 (±1.25)	1.5 (0.07)
Vehicle Control (DMSO)	8.25 (±1.2)	1.12 (0.08)
TEE (25 mg/kg)	6 (±0.91)	1.68 (±0.07)
TEE (50 mg/kg)	8.5 (±1.29)	1.47 (±0.06)
TEE (100 mg/kg)	9 (±0,81)	1.43 (±0.06)
TEE (250 mg/kg)	10.7 (±1.52)	0.95 (±0.2)
SEE (500 mg/kg)	7.5 (±1.29)	1.08 (±0.08)
SEE (1000 mg/kg)	7.4 (±1.28)	1.31 (±0.07)
SEE (2000 mg/kg)	9.6 (±1.25)	1.95 (±0.01)

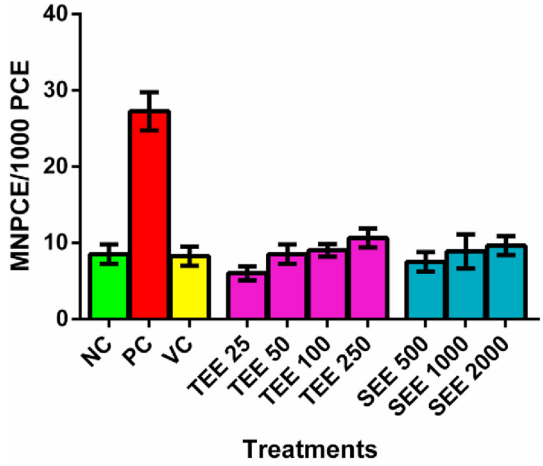

Figure 2. Assessment of Genotoxicity Index (MNPCE in 1000 PCE) in bone marrow cells of Balb/c mice treated with different concentrations of TEE an SEE obtained from *Arachis hypogaea* L. NC: negative control; PC: positive control; VC: vehicle control; TEE: tegument ethanolic extract; SEE: seed ethanolic extract.

group. This suggested that TEE and SEE from *A. hypogaea L.* did not induce damage at genomic level in the ranges of concentrations used, given the low frequency of micronuclei. In relation to the toxic capacity of the extracts, the toxicity index values (Table 1 and Figure 3) revealed non significant differences ($p > 0.05$) between the negative control

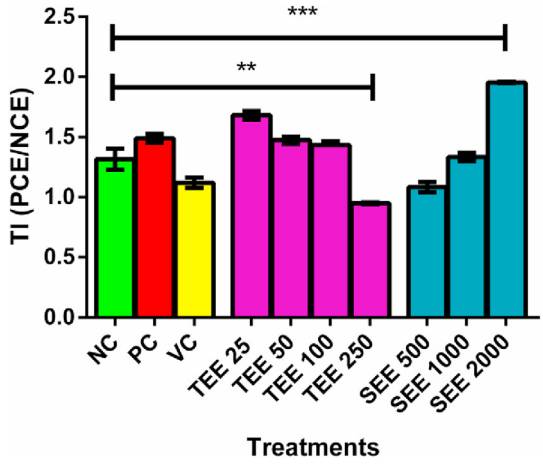

Figure 3. Assessment of Toxicity Index (PCE/NCE) in bone marrow cells of Balb/c mice treated with different concentrations of TEE an SEE obtained from *Arachis hypogaea* L. NC: negative control; PC: positive control; VC: vehicle control; TEE: tegument ethanolic extract; SEE: seed ethanolic extract.

system and the treatments of TEE (25, 50, and 100 mg/kg) and SEE (500 and 1000 mg/kg), nor with the group that received DMSO. Therefore, the evaluated concentrations of TEE and SEE from *A. hypogaea* were not toxic for the stem cells of the mouse bone marrow, with the exception of the highest concentrations of the extracts (250 mg/kg for TEE $p = 0.0058$ and 2000 mg/kg for SEE $p = 0.0003$). There were no sex-dependent changes after any treatment.

3.2.2. Comet assay

TEE and SEE did not exert genotoxic effects on blood samples of mice evaluated by the Comet assay (Figure 4).

A person of average weight with a moderate consumption of peanuts (28–42 g/day), would be ingesting less than 100 mg/kg b.w. of tegument and less than 2000 mg/kg b.w. of seeds (Jones et al., 2014). This means that the consumption of peanut is safe and does not cause acute toxicity at the concentrations evaluated. Moreover, pharmacological extract doses may be safe. The results of the present work are in concordance with Qu et al. (1992), who found that the emission of peanut oil was not genotoxic. Therefore, peanut as ethanolic extract or oil does not cause damage to DNA. There are some genotoxicity studies of other species of the family Fabaceae. Remigio-Montero et al. (2007) carried out acute toxicity studies of an ethanolic extract from *Indigofera suffruticosa* (Fabaceae) on Swiss mice. And, they demonstrated that tested concentrations of 500 and 2000 mg/kg did not induce toxicity. These results are similar to the ones obtained for SEE, which presented absence of toxicity at similar concentrations of 500 and 1000 mg/kg. These authors demonstrated certain genotoxicity, as the extract exhibited an increase in the frequency of MNPCE at the highest concentration evaluated. In contrast, TEE and SEE from *A. hypogaea* L. did not exhibit genotoxicity at any of the concentrations evaluated.

3.3. Identification of the tegument and seed of peanut compounds

The yields of TEE and SEE were 16% and 13% w/w, respectively, obtained by simple alcoholic extraction. The chemical evaluation of the

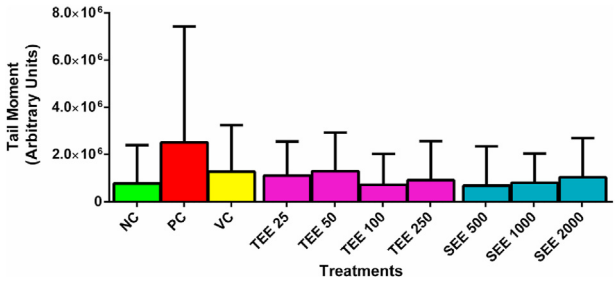

Figure 4. Comet Assay of blood samples of Balb/c mice treated with different concentrations of TEE an SEE obtained from *Arachis hypogaea* L. NC: negative control; PC: positive control; VC: vehicle control; TEE: tegument ethanolic extract; SEE: seed ethanolic extract.

A. hypogaea extracts was oriented towards the search of active compounds previously reported in peanut species (Chukwumah et al., 2012; Sarnoski et al., 2012; Alasalvar and Bolling, 2015; Ashim et al., 2015). The chemical composition analysis of TEE revealed high concentration of proanthocyanidins (O.D. 550 nm: 1.39 ± 0.15) and absence of phytosterols (Table 2). These results agree with those of Alasalvar and Bolling (2015), who also found proanthocyanidins in peanuts, and with those Sarnoski et al. (2012) Oldoni et al. (2016) and Larrauri et al. (2014), who indicated high content of this compound, especially in peanut skin.

The Folin-Ciocalteu test showed 74.33 ± 1.10 and 15.05 ± 0.06 mg GAE/g of dried extract of total phenolic content for TEE and SEE, respectively (Table 2). The statistical analysis showed that the tegument had much more phenolic compounds than the seed of the peanut ($p < 0.0001$, Figure 5). This is due to roles that different parts of the peanut play. Seeds are an important source of nutrients, the value of which lies in the storage of carbohydrates, proteins and lipids that are deposited during the development and maturation of the embryo (Courtis, 2013). On the other hand, the tegument stands out because of the presence of phenols, since these, being bioactive compounds, play an important role in the protection and conservation of the seed. Thus, the tegument constitutes the first defense of the seed against the adverse conditions of the surrounding environment, it protects it from mechanical stresses and from the invasion of pathogenic organisms (Perissé, 2002).

The concentration of total phenols in TEE is similar to that determined by Davis et al. (2010), who reported 73.44 mg GAE/g of extract in tegument of peanut.

The content of phenols in the *A. hypogaea* tegument reported by Alvarez Muñoz and Luzón Maza (2015), corresponded to 7.67% of its chemical composition. This percentage coincides with that found in the TEE, which was 7.43%.

A study made with other species of the same genus, *Arachis repens*, revealed a phenolic content of 27.26 ± 0.26 mg GAE/g of dried extract in a methanolic extract of leaves (Garcia et al., 2016). This concentration was much lower than the one found in TEE of *A. hypogaea* and higher than the concentration found in SEE in the present work.

On the other hand, Sebei et al. (2013), investigated the chemical composition of *A. hypogaea* L. seeds grown in Tunisia. The analysis of total phenols showed that seeds contained a low content of these components. The Chounfakhi variety that presented the highest content, indicated a value of 2.1 ± 0.09 mg GAE/g of extract, followed by the Massriya cultivars that had 1.35 ± 0.08 mg GAE/g of extract and, Trabilsia that presented the lowest concentration with 1 ± 0.06 mg GAE/g of extract. On the contrary, in the present study it was demonstrated that the seed from *A. hypogaea* L. contains a higher content of total phenols.

Therefore, due to the above results, the HPLC analysis was oriented towards the detection of polyphenols and flavonoids. HPLC qualitative analysis identified the presence of caffeic acid, an organic acid (Figure 6). The quantification of this compound was made from a calibration curve, which showed a 2.46% percentage. Other researchers also found caffeic acid in peanuts. Sebei et al. (2013) identified it in four types of *Arachis hypogaea* seed cultivars, in quantities similar to those in TEE. The seed from *Massriya* presented 4% and the cultivars from *Sinya, Chounfakhi* and *Trabilsia* contained 1.22%, 1.78% and 1.48% caffeic acid, respectively. Sales and Resurrección (2010) also reported the presence of this phenolic compound in peanut tegument. Similarly, Alasalvar et al. (2015) detected it in skin extract from Runner, Virginia and Spanish peanuts.

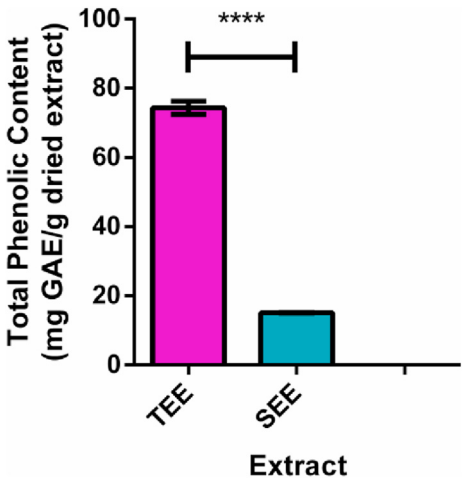

Figure 5. Total phenolic content of TEE and SEE of *A. hypogaea* L.

Caffeic acid was reported in other legumes, but in less quantity than in *A. hypogaea* L. (<1%). Telles et al. (2017) found it in ten bean varieties from genus *Phaseolus* and *Vignay*. And Misiak et al. (2017) in the methanolic extract of *Melilotus officinalis* L.

On the other hand, SEE was analyzed in the gas chromatographer. Different fatty acids were identified and quantified (Figure 7). The major component was oleic acid (9-octadecenoic acid-omega 9) since it represented 58.84% of its chemical composition. Secondly, linoleic acid (omega 6) was found in 11.31% and then hexadecanoic acid (palmitic acid) in 8.37% (see Table 3).

The fatty acids percentages were observed within the values registered by the Florapower oil factory. Peanut oils contain approximately 35–69% monounsaturated oleic acid, 13–35% difunctional linoleic acid and 8–14% palmitic acid (Florapower, 2019).

Similarly, Mahatma et al. (2016) also observed oleic, linoleic, and palmitic acids as major fatty acids and oleic acid in the range of 40–61% in the 60 genotypes of peanut.

Özcan and Seven (2003) also found that the major fatty acids were oleic, linoleic, and palmitic acids found in the seed oils of peanut from ÇOM and NC-7 cultivars (Turkey). In agreement with the present study, both varieties showed higher concentrations of oleic acid and lower concentrations of palmitic acid. They determined similar percentages in the variety ÇOM, that it presented 55.07% of oleic acid and 8.70% of palmitic acid. The linoleic acid percentage was greater in this cultivar (25.13%). Instead, NC-7 had less oleic acid (43.13%) and more of palmitic (13.03%) and linoleic (35.20%) acids.

Wang et al. (2012) analyzed the composition of fatty acids of seed oil from fifty peanut accessions in the US germplasm collection and indicated that oleic, linoleic, and palmitic acids were the main fatty acids along with stearic and behenic acids. The percentage variability of fatty acid content of the peanut accessions was between 37.0% and 55.6% for oleic acid, between 25.2% and 39.7% for linoleic acid and 8.2%–13.8% for palmitic acid. The values of oleic and palmitic acids were similar to those obtained in the present study.

In other investigation, the analysis of oils of different varieties from Creole peanut revealed a lower amount of oleic acid (35.6%). Although, the percentages of linoleic and palmitic acids were similar to those

Table 2. Chemical analysis of tegument ethanolic extract (TEE) and seed ethanolic extract (SEE) of *Arachis hypogaea* L.

Extracts of *A. hypogaea* L	Total Phenolic Content mg GAE/g dried extract	Total Proanthocyanidin Content O.D. 550 nm	Total Phytosterol Content (+/-)
TEE	74.33 ± 1.10	1.39 ± 0.15	-
SEE	15.05 ± 0.06	ND	ND

GAE: gallic acid equivalents; ND: non determined.

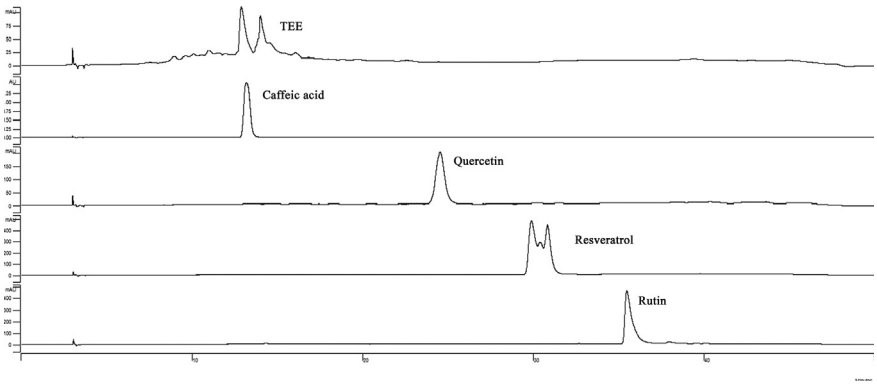

Figure 6. TEE chromatogram from *A. hypogaea* L.

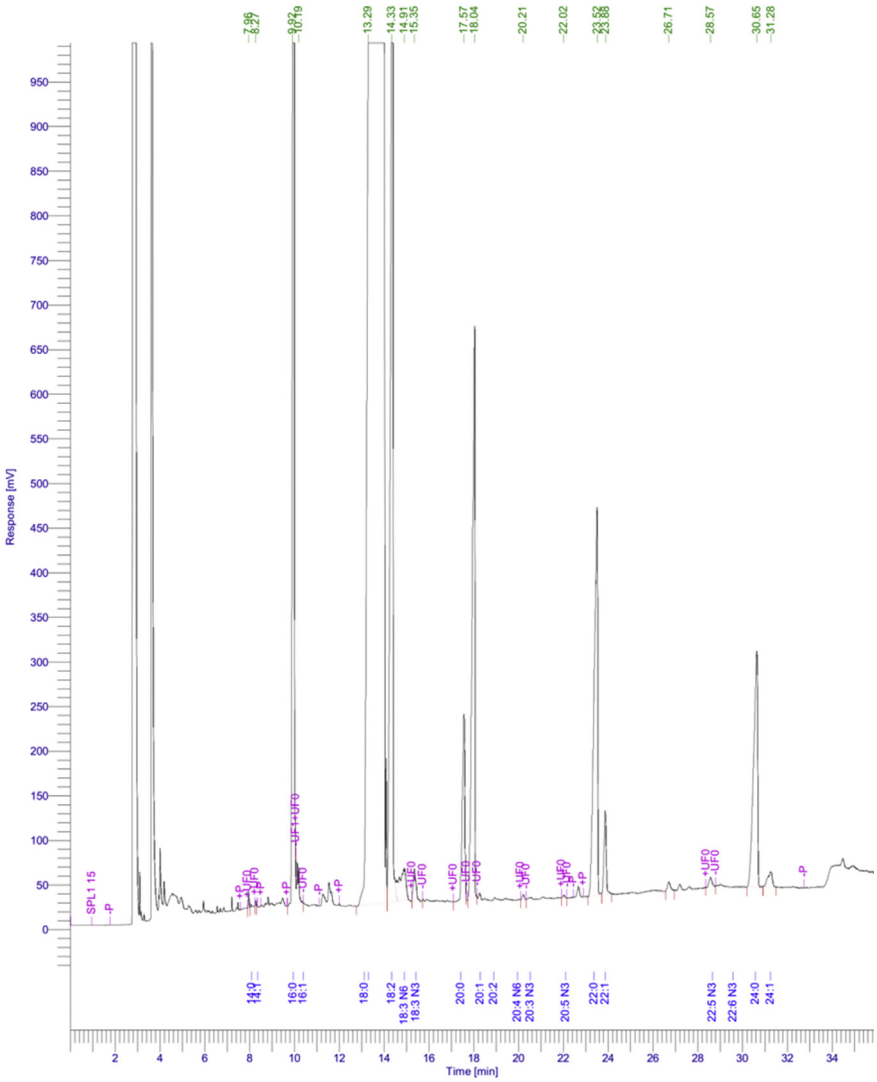

Figure 7. Fatty acids composition of SEE from *Arachis hypogaea* L., analyzed by a Clarus 500 gas chromatograph.

reported in the present study (16 and 9.6%, respectively) (Caracterización fisicoquímica del aceite de algunas variedades de cacahuate criollo, 2019).

Giuffrè et al. (2016) also reported high content of oleic acid (44.61 %–50.94 %) in oils obtained from seeds of ten Algerian peanuts even though it was a lower concentration when compared to that of the peanuts from Argentina we studied.

Thus, peanut seed is in general an important source of oleic acid. This component increases the nutritional value of the peanut seed, since its consumption can provide health benefits. For example, one of its properties is decrease the low density lipoprotein content in the

Table 3. Fatty acids detected in SEE from *A. hypogaea* L. and their respective percentages.

Fatty acids	Nomenclature	Porcentage
14:0	Myristic	0.05
14:1	Myristoleic	0.02
16:0	Palmitic	8.37
16:1 n7	Palmitoleic	0.25
18:1 n9	Oleic	58.84
18:2	Linoleic	11.31
18:3 n6	γ-Linolenic	0.95
18:3 n3	α-Linolenic	0.35
20:0	Arachidic	1.94
20:1	Eicosenoic	6.45
20:4 n6	Arachidonic	0.14
20:5 n3	Eicosapentaenoic	0.02
22:0	Behenic	5.82
22:1	Erucic	0.85
22:5 n3	Docosapentaenoic	0.11
24:0	Lignoceric	4.12
24:1 n9	Nervonic	0.41

blood, while increasing the high density lipoprotein (Giuffrè et al., 2016).

The absence of cytogenotoxicity of SEE could be explained from the chemical composition of this extract. Different studies showed that oleic, linoleic and palmitic fatty acids are neither cytotoxic nor genotoxic. For example, Chaikul et al. (2017), reported the absence of cytotoxicity of seed oil from *Camellia oleifera* (Theaceae), in which oleic and palmitic acids are the major components. Paskaleva et al. (2014), evaluated the genotoxicity of palmitic acid and its derived metabolites, by bacterial mutation, mouse lymphoma and mouse micronucleus assays and found genotoxicity absence. In the same way, Honda et al. (2016), reported genotoxicity absence of oleic and linoleic acids.

Even more, other researchers, Meddeb et al. (2018), showed that linoleic and oleic acids were responsible of the cytoprotective properties of seed oil from other plant (*Cardo mariano*) by attenuating the cytotoxicity exerted by the ketocholesterol and hydroxycholesterol compounds.

On the other hand, caffeic acid (2.46%) which is one of the major compounds of TEE from *A. hypogaea*, showed absence of genotoxicity in other researches. Bhalli et al. (2019) demonstrated that 1000 mg/kg b.w. of caffeic acid was not genotoxic using micronuclei test and comet assay. In another work, it was reported that derivatives of caffeic acid do not cause damage to genetic material in micronucleus and comet tests and have antimutagenic capacity (UNAM, 2015). With regard to the cytotoxicity studies, caffeic acid produced toxicity at high concentrations in Vero cells (with CC50 = 440.8 μg/ml) (Ordaz-Trinidad et al., 2014), indicating its low cytotoxicity.

In conclusion, the present study demonstrated that the peanut use is safe, as their seed and tegument extracts do not generate cytotoxicity or genotoxicity at concentrations that are recommended for healthy uses, such as nutrition, food technology (food functioning, nutraceuticals, etc.), and phytomedicine. Furthermore, several potentially bioactive compounds were found, making peanuts an excellent and accessible source for obtaining and using them in the development of therapeutic and preventive agents.

Declarations

Author contribution statement

Florencia Menis Candela: Performed the experiments; Wrote the paper.

Walter Fabián Giordano: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Patricia Liliana Quiroga, Laura Raquel Comini: Performed the experiments; Analyzed and interpreted the data.

Franco Matías Escobar, Fernando Mañas: Analyzed and interpreted the data.

Dardo Andrés Roma, Mariana Larrauri: Performed the experiments.

Elio Andrés Soria: Contributed reagents, materials, analysis tools or data; Wrote the paper.

María Carola Sabini: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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