Ameliorative Effect of the Oral Administration of 
*Chuquiraga spinosa* in a Murine Model of Breast Cancer Induced with 7,12-Dimethylbenz[a]anthracene (DMBA)


**Abstract**

**Objective:** To determine the ameliorative effect of the ethanolic extract of *Chuquiraga spinosa* (ChS) on 7,12-Dimethylbenz[a]anthracene (DMBA)-induced breast cancer in rats. **Methods:** 36 female Holtzman rats were divided into 6 groups. I) The negative control group received physiological saline (PS). II) ChS-200 group received 200 mg/kg of ChS. III) DMBA group was induced with DMBA (20 mg/kg) dissolved in PS and administrated orally for 15 weeks. IV) DMBA + ChS-50 group, V) DMBA + ChS-250 group, and VI) DMBA + ChS-500 group, which received the extract orally 15 weeks after DMBA induction. All data were expressed as mean and standard deviation. One-way analysis of variance (ANOVA) followed by Dunnett test was carried out to compare the mean value of different groups. Histopathological analysis was evaluated by using Image J software. **Results:** Hematoma showed that the triglyceride level was significantly lowered ($P<0.01$) and high-density lipoprotein (HDL) level was significantly increased ($P<0.01$) in groups III, IV, and V. Also, ChS extract significantly lowered the C reactive protein (CRP) level ($P<0.01$) and malondialdehyde level ($P<0.05$). There was a significant decrease in the frequency of DMBA-induced micronucleated polychromatic erythrocyte ($P<0.01$). **Conclusions:** *Chuquiraga spinosa* showed an ameliorative effect on DMBA-induced breast cancer in rats as well as antioxidant, antitumor and antigenotoxic properties.

**Key words:** Breast tumor, Preventive medicine, Phytochemical, Antioxidant, Toxicity, Anticarcinogenic agent

**INTRODUCTION**

Breast cancer is the second most common cancer in the world and in women, with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). In Peru, this cancer represents the second most frequent neoplasm, affects the adult woman and is an important cause of disease burden. It has genetic, environmental, and lifestyle factors interacting to cause this disease. Breast cancer is treated by surgery, chemotherapy, radiotherapy, alone or combined depending on the severity. The number of deaths from cancer is increasing in developing countries due to the adoption of life styles of developed countries. Epidemiological and experimental studies indicate that high consumption of vegetables helps to lower risk of breast cancer.

The inflammation plays an important role in tumorigenesis and polyphenols have a high antioxidant activity, which has been related to inflammatory activities. *Chuquiraga spinosa* (ChS) is a medicinal plant used in Yauyos (Junin-Peru). It belongs to the Asteraceae family and has a high content of flavonoids, tannins, alkaloids, terpenes and other phenolic compounds. It is used as diuretic, anti-inflammatory, renal debugger and prostate cancer.

**MATERIALS AND METHODS**

**Chemicals**

7,12-Dimethylbenz[a]anthracene (DMBA), Folin Ciocalteu reagent (FCR), gallic acid standard, minimal essential medium, fetal bovine serum, Dulbecco’s modified Eagle’s medium, dimethyl sulfoxide (DMSO), sulforhodamine B (SRB) and 5-flurouracil were obtained from Sigma Chemical Co. Kits for hematology and biochemical test were purchased from Quimica Suiza S.A. Other reagents were of chemical grade for analysis.

**Cell lines**

The cell lines MCF-7 (cancer) and 3T3 (mouse embryonic fibroblasts) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).
Preparation of the extract

*Chuquiraga spinosa* Lessing was collected in Huancayo, Junín, Peru. Taxonomic identification of the plant was carried out at the Museum of Natural History of Lima, Peru. The aerial parts were selected from the intact plants and dried at 40°C. Next, it was macerated with 96% ethanol and evaporated at 40°C. The resulting dry residue was kept in amber bottles at 5°C in darkness for later reconstitution.

Qualitative phytochemical screening

**Determination of alkaloids**

Dragendorff’s test: Dragendorff reagent was added to the extract. The presence of alkaloid was indicated by the formation of a reddish-brown precipitate.11 Mayer’s test: Mayer’s reagent was added to extract solution. The presence of alkaloid was indicated by the formation of a creamy white precipitate.11

**Determination of anthraquinones**

Bornträger test: A small quantity of chloroform and NH₄OH drops were mixed with a small amount of the extract. The presence of anthraquinone derivatives was indicated by the formation of pink, red, or violet color.11

**Determination of flavonoids**

Shinoda’s test: HCl drops and magnesium were added and mixed with the extract. The presence of flavonoids was indicated by the formation of red color.

**Determination of phenols**

Ferric chloride test: FeCl₃ was added in drops to the extract. The presence of tannins compounds was indicated by the appearance of blue-green color in the solution.11

**Determination of saponins**

Frothing test: The powdered extract and water were vigorously shaken in a test tube. The presence of saponins was indicated by the frothing.11

**Determination of steroids and terpenes**

Liebmann–Burchard’s test: 2 mL of acetic anhydrous was added to 0.2 gr of the extract and the solution was cooled while H₂SO₄ was added carefully. The presence of steroids and terpenes was indicated by the color change from violet to blue or bluish-green.11

**Determination of tannins**

Gelatin test: A 1% gelatin solution containing 10% sodium chloride was added to each leaf and bark (mixture) extract. The presence of tannins and phenolic compounds was indicated by the precipitation of a precipitate.12

Quantitative phenolic analysis

The phytochemical screening of the ethanolic extract was carried out in the facilities of the Laboratory of Experimental Pharmacology of the Faculty of Medicine (Universidad Nacional Mayor de San Marcos). The determination of phenolic compounds was done using the Folin-Ciocalteu method.13

Animals

Thirty-six female Holtzman rats were purchased from the National Institute of Health of Peru. The animals were maintained in a temperature-controlled room with alternating 12 h light/12 h dark cycles in an animal facility at the Universidad Nacional Mayor de San Marcos. Animals were fed standard rat chow and allowed free access to water. After a preconditioning period of 7 days, the experiment began. Rats were weighted and palpated to detect any mammary tumors every week.

Tumor induction

Tumor induction was performed by an intraperitoneal injection of 20 mg/Kg of DMBA. This method was developed by Barroso et al. and Arroyo et al.14,15 All groups received tumor induction, except PS and ChS200 groups.

Experimental design

Thirty-six female Holtzman rats were divided into 6 groups. The negative group, PS, received 2 mL / kg of physiological saline orally for 15 weeks. Group ChS 200 received 200 mg/kg body weight of ethanolic extract of *Chuquiraga spinosa* orally as drug control for 15 weeks. Experimental groups were induced with DMBA on the first day and received 2 mL/kg of physiological saline orally for 15 weeks. The groups DMBA + ChS50, DMBA + ChS250, and DMBA + ChS500 received ethanolic extract of *Chuquiraga spinosa* (50, 250, and 500 mg/kg body weight, respectively) orally for 15 weeks after DMBA induction.

Animals were sacrificed with pentobarbital (100 mg/kg). Blood samples were obtained to assess the biochemical parameters, hematological indicators, and nuclear abnormalities. Breast, Kidneys, liver, heart, and lungs were removed and carefully examined. The organs were fixed in 10% phosphate-buffered formalin and stained with hematoxylin and eosin (H&E).

Micronucleus test

The antigenotoxic effect was determined by the frequency of micronucleated polychromatic erythrocytes (MNPCe) proposed by Hayashi.16 Peripheral blood was obtained to prepare a blood film. They were fixed with methanol and stained with 3% Giemsa. The frequency of MNPCE in 1000 polychromatic erythrocytes (PCE) per animal, was determined to determine the levels of C-Reactive Protein (CRP).17,18

Biochemical parameters

SOD was assayed reduction of nitroblue tetrazolium (NBT) to water-insoluble blue formazan. This method was described by Beauchamp and Fridovich. According to the method of Begue and Aust, lipid peroxidation was detected by the determination of MDA production. Biochemistry VITROS and Integrated system VITROS 5600 were used to determine the total cholesterol level.19 The HDL-cholesterol level was used to determine the blood glucose. The modified Schonewille´s method estimated the total cholesterol level.20 The level of triglycerides was estimated according to Deori method.21 Mohan and Cook methods were used to determine the Alanine aminotransferase (ALT) level.22 The method of Talageri et al.23 was used to assess the Alkaline phosphatase (ALP) activity. The cleavage of urea with urease (Berthelot’s reaction) was used to determine Urea according to Liu et al.23

Cytotoxicity assay in MCF-7 cell line

This *in vitro*-test was developed on tumor cell line of breast cancer (MCF-7)., and 3T3 (mouse embryonic fibroblast) was used as control normal cells. Cells were maintained at 37°C in a 5% CO₂ atmosphere. MCF-7 cell line was grown in minimal essential medium in the presence of DMEM supplemented with 10% fetal bovine serum.
of 10% fetal bovine serum and 50 μg/mL gentamicin as antibiotic to avoid any microbial contamination. 3T3 cells were grown in Dulbecco’s modified Eagle’s medium. Next, both cell lines were washed in 3 × 4 mL Hank’s balanced salt solution. Subsequently, 1 mL trypsin/EDTA was added and 10 minutes later, it was washed. The cell cultures were incubated for 8 minutes at 37°C and each culture was resuspended in 2 mL medium. Then, cells were counted using a hemocytometer.

Extract and 5-FU was diluted in dimethyl sulfoxide (DMSO) and centrifuged at 13,500 g for 10 minutes. The initial concentration used was 250 ug/mL for 5-FU and 250 ug/mL for extract. Plates were incubated for 48 additional hours. To evaluate antitumor activity, cytotoxicity bioassay method with sulforhodamine B (SRB) was performed as described by Skehan et al.25 The 50% inhibitory concentration (IC₅₀) was found by linear regression analysis. The extract selectivity index was defined as the ratio of cytotoxicity between 3T3 cell line and MCF-7 cells, which was > 1 when the cytotoxicity for tumor cells was greater than in normal cells.

Statistical analysis. Data was expressed ± standard deviation (SD). Data were analyzed for homogeneity of variance and normality by the Levene and Wilk–Shapiro tests. One-way analysis of variance (ANOVA) followed by Dunnett’s test was carried out to compare the mean value of different groups. A P-value of <0.05 was considered statistically significant in all cases. SPSS v. 20 was used to analyze the data.

Ethical considerations. The protocol was approved by the Institute for Ethics in Health of Universidad Nacional Mayor de San Marcos (00531-R-15-UNMSM). Ethical principles for research were respected and the rats were euthanized according to Recommendations for Euthanasia of Experimental Animals of the European Commission.26

### RESULTS

Table 1 shows the phytochemical analysis of the ethanolic extract of *Chuquiraga spinosa* aerial parts. In the quantitative analysis of total phenolic compounds, the extract has 20.4 ± 1 mg GAE/ g.

The results also showed that groups treated with 50, 250, and 500 mg/kg body weight extract had an increased body weight. A significant increase in body weight was found in ChS500 + DMBA group (230.00 ±20 g; *P*<0.01; compared with DMBA group). ChS50 + DMBA and ChS250 + DMBA groups also were effective during the experiment and presented significant values (218.00±10 g, *P*<0.05; 225.20±15 g; *P*<0.01, respectively) compared with DMBA group (Figure 1).

The hematological and biochemical analysis (Table 2) showed that there was no significant difference in the hemoglobin, hematocrit, cholesterol and urea levels. However, a significant reduction in the white blood cell count was observed, with a dose-dependent effect in the groups treated with ChS. Likewise, in group ChS 200, there was an increase in HDL levels and alkaline phosphatase levels. As well as a significant decrease in triglyceride levels, glucose, creatinine and transaminases. The same was observed in groups DMBA + ChS50, DMBA + ChS250, and DMBA + ChS500.

The antitumor and antigenotoxic effect of the ethanolic extract is dose-dependent. We observed significantly reduction in the mass of tumors and micronuclei in the groups treated with *Chuquiraga spinosa* compared with DMBA group (Table 3). The antioxidant activity *in vivo* as well as oxidative stress markers indicate significantly increased CRP (Figure 2) levels in DMBA group compared to the groups treated with the extract (*p*<0.05). SOD activity was significantly elevated in groups treated with ChS compared to group DMBA (Figure 3).

**Table 1: Phytochemical constituents of the ethanolic extract of *Chuquiraga spinosa* aerial parts.**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda</td>
<td>+</td>
</tr>
<tr>
<td>Quinone</td>
<td>Bornträger</td>
<td>-</td>
</tr>
<tr>
<td>Phenols compounds</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>frothing</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes and steroids</td>
<td>Liebermann–Burchard</td>
<td>+</td>
</tr>
</tbody>
</table>

Notes: (+) positive, (-) negative.
Arroyo-Acevedo, et al.: Ameliorative Effect of the Oral Administration of *Chuquiraga spinosa* in a Murine Model of Breast Cancer Induced with 7,12-Dimethylbenz[a]anthracene (DMBA)

**Table 2:** Hematological and biochemical parameters in female rats with breast cancer induced with DMBA.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>Leukocytes (10⁶ Cells/mL)</th>
<th>Cholesterol (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>Tryglicerides (mg/dL)</th>
<th>Glucosa (mg/dL)</th>
<th>Urea (IU/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>ALT (IU/dL)</th>
<th>ALKPhosphatase (IU/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>11.8 ± 0.4</td>
<td>37.2 ± 0.8</td>
<td>7.8 ± 1.7</td>
<td>115.5 ± 2.6</td>
<td>39.3 ± 1.2</td>
<td>94.0 ± 3.5</td>
<td>82.7 ± 10.4</td>
<td>18.5 ± 2.1</td>
<td>1.0 ± 0.2</td>
<td>18.3 ± 3.3</td>
<td>8.5 ± 1.3³</td>
</tr>
<tr>
<td>ChS 200mg/kg</td>
<td>11.9 ± 0.8</td>
<td>37.2 ± 3.4</td>
<td>7.5 ± 1.2</td>
<td>139.4 ± 11.5</td>
<td>57.6 ± 3.8⁴</td>
<td>103.0 ± 7.9</td>
<td>60.2 ± 1.4⁴</td>
<td>20.0 ± 2.7</td>
<td>0.8 ± 0.0</td>
<td>10.2 ± 1.4⁴</td>
<td>106.2 ± 3.0⁴</td>
</tr>
<tr>
<td>DMBA</td>
<td>10.6 ± 0.7</td>
<td>32.2 ± 1.9</td>
<td>10.4 ± 1.2</td>
<td>148.0 ± 7.4</td>
<td>30.7 ± 2.9</td>
<td>164.3 ± 10.8</td>
<td>100.0 ± 3.5</td>
<td>28.0 ± 3.6</td>
<td>99.5 ± 3.2</td>
<td>25.8 ± 1.9⁴</td>
<td>27.2 ± 3.4⁴</td>
</tr>
<tr>
<td>DMBA + ChS 50</td>
<td>10.6 ± 0.9</td>
<td>33.0 ± 2.3</td>
<td>6.2 ± 1.3</td>
<td>135.0 ± 4.3</td>
<td>51.4 ± 6.3</td>
<td>78.8 ± 9.9</td>
<td>67.8 ± 8.0⁴</td>
<td>15.8 ± 2.0</td>
<td>86.6 ± 4.4</td>
<td>18.4 ± 2.6</td>
<td>21.0 ± 5.2⁴</td>
</tr>
<tr>
<td>DMBA + ChS 250</td>
<td>11.5 ± 1.1</td>
<td>36.6 ± 3.2</td>
<td>6.3 ± 0.9</td>
<td>126.4 ± 5.4</td>
<td>50.6 ± 4.5</td>
<td>79.4 ± 4.7</td>
<td>59.9 ± 10.6</td>
<td>15.9 ± 0.9</td>
<td>84.0 ± 5.3</td>
<td>20.4 ± 2.7</td>
<td>12.4 ± 1.0⁴</td>
</tr>
<tr>
<td>DMBA + ChS 500</td>
<td>11.6 ± 0.4</td>
<td>37.3 ± 0.9</td>
<td>4.9 ± 0.6</td>
<td>110.5 ± 9.3</td>
<td>52.5 ± 5.7</td>
<td>94.8 ± 3.1</td>
<td>59.1 ± 13.3</td>
<td>19.0 ± 0.6</td>
<td>81.0 ± 6.8</td>
<td>16.5 ± 2.5</td>
<td>23.0 ± 4.7⁴</td>
</tr>
</tbody>
</table>

Notes: PS: Physiological saline; Hb: hemoglobin; Hct: hematocrit; HDL: High density lipoprotein; ALT: Alanine Aminotransferase. ANOVA, Dunnett’s test a: p<0.05 (Compared with DMBA), b: p<0.05 (Compared with ChS).

**Table 3:** Evaluation of tumor masses and micronucleus in rats with breast cancer induced by DMBA.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Tumor mass (X)</th>
<th>Micronucleus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>0.0 ± 0.0³</td>
<td>0.0 ± 0.0³</td>
</tr>
<tr>
<td>ChS 200mg/kg</td>
<td>0.0 ± 0.0³</td>
<td>0.0 ± 0.0³</td>
</tr>
<tr>
<td>DMBA</td>
<td>2.5 ± 0.5</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>DMBA + ChS 50</td>
<td>0.2 ± 0.4⁴</td>
<td>1.4 ± 0.9⁴</td>
</tr>
<tr>
<td>DMBA + ChS 250</td>
<td>0.2 ± 0.4⁴</td>
<td>0.6 ± 1.3⁴</td>
</tr>
<tr>
<td>DMBA + ChS 500</td>
<td>0.0 ± 0.0³</td>
<td>0.5 ± 0.6⁴</td>
</tr>
</tbody>
</table>

ANOVA, Dunnett’s Test *: p<0.05 (Compared with DMBA)

The rat mammary glands were observed under the microscope (Figure 4). All groups treated with DMBA presented adenocarcinoma. This coincides with that evaluated in the anatomopathological study, where the presence of intraductal adenocarcinoma is observed in group DMBA. It was observed desmoplastic reactions, metaplasia and cell invasion in the groups treated with *Chuquiraga spinosa*.

**DISCUSSION**

Cancer is the first cause of death in the world. Breast cancer represents the second most common cancer to be diagnosed and incidence in developing countries is progressing.⁹ Inflammation plays an important role in the development of cancer.⁹ DMBA is a poly cyclic aromatic hydrocarbon potent that induces DNA damage [¹⁴] and cell proliferation in breast tissue.¹

The ethanolic extract of *Chuquiraga spinosa* presents various secondary metabolites with high antioxidant activity (Table 1). These results are consistent with those reported by Landa et al.²⁷ who detected 9 flavones glycosides,²⁸ which are associated in retarding cell migration in breast cancer cells by inhibiting P53-ROS ²⁹ pathway. Its phenolic compounds possess antioxidant activity, by chelation of metals. Their anti-proliferative effect and induction of apoptosis in tumor cells has been observed.¹ Also, the triterpenes are associated with high antiproliferative activity.³⁰ Cancer cells have and increased amount of...
reactive oxygen species and free radicals. The presence of antioxidants in vivo is a pharmacological target for cancer therapy. Terpenes are anti-inflammatory compounds, while casearina G could be more useful for the formulation of antitumor drugs breast and prostate.

Among the associated factors of breast cancer, it is estimated that chronic inflammation triggers the alteration of macrophages. High CRP levels are associated with low survival in patients with breast cancer, this coincides with our results (Figure 2). ChS reduced CRP levels dose-dependent and showed anti-inflammatory activity. There is growing evidence that obesity is associated with breast cancer in early stages. Concordantly, we observed the increase in triglycerides, cholesterol and reduced HDL (Table 2) in DMBA group and reduction in the groups treated with ChS. Nevertheless, it is observed an increase in glycemia levels of group DMBA and a potent hypoglycemic effect in ChS 200 (Table 2).

Ch. spinosa extract showed cytotoxic activity in vitro against the MCF-7 tumor cell line, exceeding 5-FU activity (Table 4). However, the cytotoxicity for the 3T3 cell line was minor. It also showed a good security profile by having selectivity rates above unity. Similarly, the degree of dose–effect relationship was significant in all the cell lines.
The ethanolic extract of *Ch. spinosa* presented a selectivity index of 6.02 compared to 0.0037 with 5-FU. Studies with silymarin showed that the alterations in the progression of the cell cycle could be responsible for its anticarcinogenic effect on MCF-7 cells. Resveratrol, a polyphenol, inhibits prostate cancer growth and alters mitogenesis. It achieved significant inhibition of the proliferation of MCF-7.26

Thus, the *Chuquiraga spinosa* triggers reduces breast cancer, possessing powerful antioxidant and anti-inflammatory activity in vivo. As well as the hypoglycemic and lipid-lowering effect. So, the ethanolic extract *Chuquiraga spinosa* is attributed antitumor effects on DMBA - induced in female rats with breast cancer.

**CONCLUSIONS**

From the above results, it can be inferred that *Chuquiraga spinosa* has high levels of phenolic compounds. *Chuquiraga spinosa* extract demonstrated antioxidant, antitumorogenic and antigenotoxic properties and we conclude that it can have an ameliorative effect on DMBA-induced breast cancer in rats.

**ACKNOWLEDGEMENT**

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**DISCLOSURE STATEMENT**

The authors have no conflicts of interest to declare.

**FUNDING SOURCES**

Nil.

**REFERENCES**


**GRAPHICAL ABSTRACT**