



# Chemopreventive effect of *Callistemon citrinus* (Curtis) Skeels against colon cancer induced by 1,2-dimethylhydrazine in rats

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## Abstract

**Purpose** *Callistemon citrinus* (Curtis) Skeels is a shrub native of Australia. In spite of containing an important number of bioactive compounds (1,8-cineole, limonene and  $\alpha$ -terpineol) recognized as a potential chemotherapeutic agents, it is only used as an ornamental plant in Mexico. This study investigated the chemopreventive effect of *C. citrinus* leaves extract on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in rats.

**Methods** Twenty-four rats were divided into 3 groups of eight rats. Group 1 served as negative control, groups 2 and 3 were given subcutaneous injections of DMH (65 mg/kg b.w.) twice a week the first 2 weeks, and then one the third week. In addition, group 3 was administrated with leaves extracts (250 mg/kg b.w., orally daily) during the 22 weeks of the experiment. Animals were killed and the presence of colon tumors and aberrant crypt foci (ACF) were scored for number and distribution pattern along the colon. The activity of two-phase II enzymes quinone reductase (QR) and glutathione *S*-transferase (GST) was determined in the liver and three segments of the colon: proximal, middle and distal.

**Results** The results show that rats feed with *C. citrinus* leaves extract significantly reduced the size of tumors, the number of ACF and the crypt multiplicity. Additionally, *C. citrinus* leaves extract increased or maintained the activity of QR and GST in the different tissues as compared with DHM-treated group ( $p > 0.05$ ).

**Conclusion** This study demonstrates that *Callistemon citrinus* extract could have a chemopreventive effect against colon carcinogenesis.

**Keywords** 1,2-Dimethylhydrazine · Colon cancer · Chemoprevention · Quinone reductase · Glutathione *S*-transferase

## Introduction

Colorectal cancer (CRC) is the third most diagnosed type of cancer in the world (Araújo et al. 2011). The increase of life expectancy causes an increase of CRC (Pereira et al. 2017).

It is well known that a balanced diet can prevent this type of cancer, while one deficient in fiber and fruits increases the risk of developing it. (Begleiter et al. 2003). Some dietary compounds, mostly plant derived may prevent the initiation of carcinogenesis, inactivate or remove mutagens or could

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inhibit cancer progression by detoxification or by direct inhibition of tumor progression. (Begleiter et al. 2003; Cooper et al. 2010).

Phase I and II detoxification enzymes protect the body against damage caused by carcinogenesis, mutagenesis, and drugs. There are reports suggesting that their induction, particularly phase II enzymes, help to prevent cancer (Hong and Sporn 1997; Tan and Spivack 2009). The use of plants to protect against disease has been employed since ancient times. A more recent example was provided by Hafez et al. (2016), they reported that *Cichorium endivia* L. showed a protective effect against colon cancer.

*Callistemon citrinus* (Curtis) Skeels (syn: *Callistemon citrinus* var. *splendens* Stapf), also named Crimson bottlebrush (<http://www.theplantlist.org/>) (Family: Myrtaceae), is an Australia native shrub, widely cultivated around the world (Cock 2008). Its ethnopharmacological use has been to treat cough, pain, gastrointestinal disorders, and as an anti-inflammatory agent (Goyal et al. 2012; Cao et al. 2017). In Mexico, *C. citrinus* is only used as an ornamental tree (Petronilho et al. 2013).

Phytochemical studies have shown that *C. citrinus* is a rich source of bioactive compounds including polyphenols and terpenoids (Jeong et al. 2009; Petronilho et al. 2013) with many biological activities, such as a high antioxidant capacity (Shukla et al. 2012). 1,8-Cineole, limonene, terpinolene, linalool, geraniol, and phytol have been reported as major constituents of the essential oils (Oyededeji et al. 2009; Petronilho et al. 2013).

In spite of its high content of bioactive compounds and multiple pharmacological activities, there are no reports about the possible effect of this plant on preventing oxidative damage on an in vivo cancer model. Therefore, this study was carried out to evaluate the chemopreventive effect of *C. citrinus* leaves extract against colorectal cancer induction in Wistar rats.

## Materials and methods

### Biological material

Leaves from *Callistemon citrinus* (Curtis) Skeels were collected in March 2015, at the central campus of Michoacan University of Saint Nicolas of Hidalgo (UMSNH) in Morelia, Mexico (19° 41' 11.3" N latitude and 101° 12' 18.4" O longitude, average altitude 1920 m). A voucher specimen was deposited in the EBUM Herbarium (UMSNH) under the registration number EBUM23538.

### Preparation of ethanolic extracts

Fresh leaves were cleaned, weighed and macerated with 96% ethanol in a 1:10 ratio (1 g/10 mL of ethanol). The extract was allowed to stand in the dark at room temperature for 5 days. Subsequently, they were dried by evaporation with vacuum removal in a rotary evaporator at 45 °C and were stored at 4 °C protected from light until their later use. The ethanolic extract has been characterized by GC–MS as reported by Petronilho et al. (2013).

### Animals

Male Wistar rats were used and maintained under standard conditions: 12 h of light/12 h of darkness, relative humidity of 60–70%, and temperature of 20 °C. They were fed ad libitum with free access to food and water. The animals had an age of 8 weeks and average weight of 200 g at the beginning of the experiment. They were kept in the bioterium of the Biological Chemistry Research Institute/UMSNH. The attention of the animals and the experimental process were carried out according to the guide for the care and use of laboratory animals, of the Official Mexican Norm (NOM-062-ZOO-1999) (Mexicana 1999).

### Cancer induction model

The method of induction of colorectal cancer with 1,2-dimethylhydrazine (DMH) modified from Jucá et al. (2014) was used. Twenty-four healthy rats were randomly distributed into 3 groups ( $n=8$ ).

Group 1 served as a normal group (negative control), and received the vehicle solution.

Groups 2 and 3 (DMH and DMH + leaves extract, respectively) were administered with five subcutaneous injections of an aqueous solution of 1,2-dimethylhydrazine (DMH) at a dose of 65 mg/kg b.w. The injections were administered twice a week for 2 weeks and once the third week of the experiment. The solution was prepared by dissolving the DMH in a 0.9% NaCl solution with 1.5% EDTA pH of 6.5.

Additionally, on the first day of the experiment, group 3 received leaves extract by orally gavage at a daily dose of 250 mg/kg b.w., this dose was selected on the basis of our previous work with *C. citrinus* hepatoprotective effect (Lopez-Mejia et al. 2016). The vehicle solution was commercial corn oil Mazola®. Water and food were administered ad libitum. The weight was recorded to adjust the doses of the extracts. The total experimental duration was 22 weeks. At the end, all the individuals were killed by overdose of sodium pentobarbital (150 mg/kg b.w.). Blood samples were also taken by cardiac puncture, placed in tubes without

anticoagulant and, after 1 h, they were centrifuged at 4 °C and 3000 rpm for 20 min to obtain serum. After this process, the samples were stored at –20 °C.

### Histological study

Animals were dissected through a middle thoracic-abdominal incision to look for evidence of visible tumors in the internal organs. Subsequently, the large intestine and liver were removed. The intestine was opened longitudinally and both organs were washed with physiological solution (NaCl 0.9%). The presence of tumors in colon was observed and recorded. Colon and liver samples were frozen immediately at –20 °C for subsequent enzymatic determinations.

Samples of tumors and no tumors areas of the colon were taken to perform a histopathological study. The samples were fixed in a formalin solution (10%) for 48 h. Subsequently, they were embedded in paraffin and transverse sections with a thickness of 7 µm were made with the aid of a microtome. After that, the samples were stained by hematoxylin–eosin method and observed under a microscope.

### Analysis of aberrant crypt foci (ACF)

The study of ACF was carried according to Piña (2007) and Roncucci et al. (1991). Colon was measured and divided into three different portions: proximal, middle and distal in relation to cecum. Random fragments of 1 cm from different zones were cut and placed on filter paper and fixed in formaldehyde (4%) for 24 h. Later, they were stained with 0.2% methylene blue solution and observed under a microscope (10×). The ACF are characterized by having a thickened epithelial rim, presenting laminar openings and being of greater evident size than the crypts that surround them. The number of ACF in each focus was recorded to determine crypt multiplicity.

### Preparation of tissue homogenates

0.25 g of liver, proximal, middle and distal zone colon was homogenized adding 1 mL of a 0.1 M phosphate buffer solution with 1% SDS pH 7.4. They were placed in Eppendorf tubes and centrifuged twice: the first time for 20 min at 13,000 rpm (4 °C) and the second time for 5 min at the same speed and temperature. The supernatant was recovered, frozen and stored at –20 °C for biochemical estimation.

### Determination of phase II enzyme activity

Total glutathione *S*-transferase (GST) and quinone reductase (QR) activities were measured in proximal, medium and distal colonic and hepatic cytosolic fractions.

The GST activity was measured by the method of Habig et al. (1974) and modified by Pour et al. (2014). The reaction mixture consisted of 980 µL of 0.1 M phosphate buffer (pH 6.5), 10 µL of 10 mM reduced glutathione and 10 µL of 100 mM 1-chloro-2,4-dinitrobenzene (CDNB) used as substrate. 900 µL of this solution was taken and 100 µL of sample was added for a final volume of 1 mL. Samples were incubated at 30 °C for 5 min. Blank tube was the reaction mixture without the enzyme (sample). The changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as nmoles of GSH-CDNB conjugate formed/min/mg protein using a molar extinction coefficient of  $9.6 \times 10^3$  M/cm.

QR activity was measured according to Perez et al. (2009). Incubation mixture (1 mL) contained 25 mM Tris/HCl pH 7.5; 0.18 mg/mL BSA; 5 µM FAD; 0.2 mM NAD(P)H; 40 µM 2,6 dichloroindophenol (DCPIP) and 10 µL of tissue homogenate. The blank tube contained complete assay mixture without the sample, and 0.2 mM NAD(P)H is used for calculation of enzyme activities. Control tube contained complete assay mixture without the enzyme, was included for background correction. The absorbance was measured at 600 nm. Enzyme-specific activity was expressed as nmol DCPIP reduced/per min per mg protein.

### Proteins and albumin determination

Proteins concentration was measured according to Bradford (1976) and albumin by the method of Gendler (1984). Samples were measured at 590 nm by spectrophotometry and bovine serum albumin (BSA) was used as standard (1 mg/mL and 80 mg/mL, respectively).

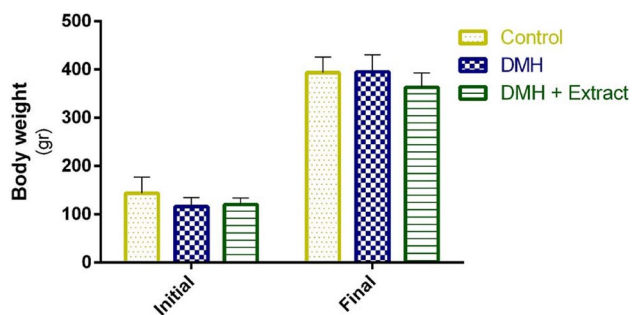
### Statistical analysis

Each organ homogenate represented one sample from individual rat; all assays were performed in triplicates. ANOVA and Tukey's test were used to assess the significance of the data. *p* values of *p* < 0.05 were considered significant.

## Results and discussion

### Body weight

Figure 1 shows the average growth rate of the animal after 22 weeks of DMH induction. Food and water intake were similar in the three groups. This indicates that DMH and *C. citrinus* leaves extract did not suppress the calorie intake. Although the differences in weight between groups, they were not statistically significant. Similar results were observed in animal weight at 10 weeks (Shwter et al. 2014) or 30 weeks (Manju and Nalini 2007).



**Fig. 1** Body weight changes between treatments. No statistical differences were found (Anova,  $p \leq 0.05$ )

### Colorectal cancer induction model and tumor formation

The colonic carcinogenesis model was successfully established. All animals in the group treated only with the carcinogen DMH developed tumors in the large intestine (Fig. 2). No tumor was found in vehicle-treated animals (control) and no tumors were visible in any other organ in any of the groups. During the period of stabilization and acclimation, two rats of each group died; therefore, these deaths are not attributable to the toxic effect of DMH.

The group treated with the carcinogen (DMH) evidenced the formation of a total of 26 tumors predominating in the middle area with 13 total tumors (50%). The average number

of tumors per rat in this group was of 4.3 and there was presence of them in all the individuals. In the group with CRC induced but administered with the leaves extract, four of the six individuals presented tumor. In this case, the total number of tumors was of 27, with an average of 4.5 tumors per rat; however, the tumor size was smaller than of the DMH-treated rats (Table 1).

The middle area of the colon presented more tumors, followed by distal colon and finally the proximal colon. There was no difference in the distribution of tumors among the groups. These data are in agreement with those reported in the literature, where the incidence of tumors is always lower in the proximal zone (Li and Li 2005; Jucá et al. 2014). Although the number of tumors was similar in both groups, the effect of the extract was observed in tumor volume. Table 1 shows the inhibitory effect over the tumor growth. 1,8-Cineole presents a high capacity to inhibit tumor growth in mice (Murata et al. 2013). In a previous work, we reported the main compound in *C. citrinus* leaves is 1,8-cineole (Petronilho et al. 2013). Therefore, the effect on the tumor size observed in this study is attributed to this compound.

### Histopathological evaluation

There are many reports that show crypt proliferation and dysplasia in the colonic mucosal epithelium when DMH-induced colon carcinogenesis in a rat model. The histopathology study of colon tissue section in the control group,

**Fig. 2** Representative image of colonic carcinogenesis model: **a** colon of an individual of the control group with no tumors observed; **b** colon of one of the individuals of the group treated only with the carcinogen; **c** colon of one individual from the group treated with the DMH and *C. citrinus* extract. Tumors are enclosed in yellow circles



**Table 1** Effect of *Callistemon citrinus* extract (*C.c*) on tumors incidence and volume of tumor induced by DMH in rat colon after 22 weeks

	T.M	Tumor per zone			Volume of tumors per zone (cm <sup>3</sup> )			T.T
		Proximal	Middle	Distal	Proximal	Middle	Distal	
DMH	4.3 ± 0.24	4 ± 0.44	13 ± 0.75	9 ± 0.54	1.27 ± 0.31*	0.78 ± 0.15*	0.87 ± 0.23*	26
DMH + <i>C.c</i>	4.5 ± 0.14	4 ± 0.44	14 ± 0.89	9 ± 0.75	0.49 ± 0.25	0.30 ± 0.06	0.37 ± 0.23	27

Values are mean ± SD (\* $p \leq 0.05$ ,  $n = 6$ , Tukey test)

T.M tumor multiplicity (is the average number of tumors per rat), T.T total number of tumors

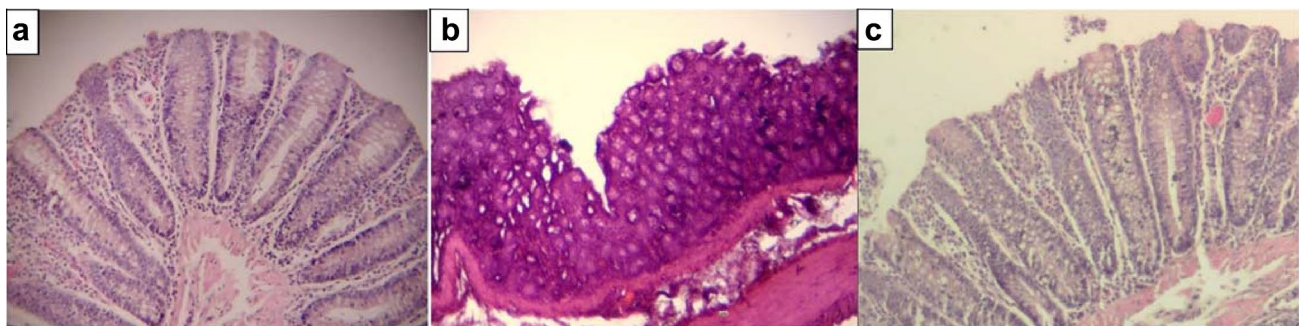


and the *C. citrinus* extract-treated rats showed normal histological structure of the mucosa, whereas in the DMH-treated rats showed different grades of dysplasia and hyperplasia (Fig. 3). These findings are in agreement with those reported by Ansil et al. (2013) and Reynoso-Camacho et al. (2015). Besides, Murata et al. (2013) reported that 1,8-cineole suppressed human colon cancer proliferation by inducing apoptosis. Murthy et al. (2012) demonstrated anti-angiogenic activity of D-limonene. A previous study reported that *C. citrinus* leaves extract is rich in terpenoids compounds (Petronilho et al. 2013). The influence of these compounds could be the reason of the observed decrease of tumor size and the minor damage produced by the DMH in the colon.

### Aberrant crypt foci

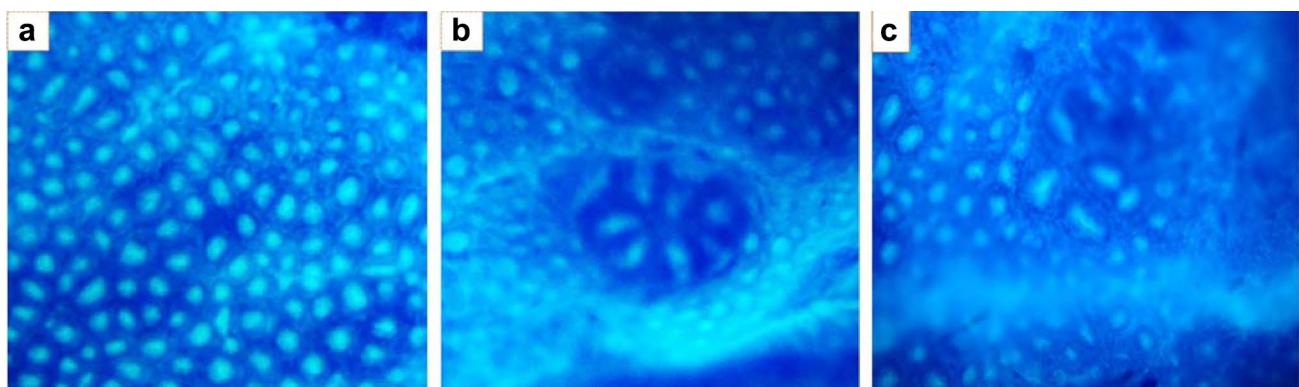
Mori et al. (2005) reported that ACF appear in a relatively short time after carcinogen administration and they continue for a long period of time in the bowel mucosa, some of them without morphological changes while others can degenerate. The presence of ACF was evaluated in the three segments of the colon of all the experimental groups (Fig. 4). The ACF

have been used as biomarkers to evaluate the initiation and promotion of chemically induced carcinogenesis (Fontana et al. 2001; Perše and Cerar 2011). ACF are considered pre-neoplastic lesions, and there are many reports about regulating the incidence of total ACF or ACF-displaying features as size, crypt multiplicity and dysplasia. (Bird and Good 2000). In our study, no ACF were observed in the control group. The DMH group had a higher incidence of ACF with larger crypts than the group treated with *C. citrinus* extract. In this group, 36 total ACF were recorded in the three areas of the colon, predominating in the distal zone (50%) (Table 2). In contrast, the animals treated with leaves extract showed a less number of ACF than DMH-treated group (15 against 36, respectively). In this case, the major number of ACF was also found in the distal zone (80% of all the ACF). Similar to control group, no ACF were observed in the proximal zone. Numerous studies mention that ACF present a randomly distribution in the different parts of the colon (Raju 2008; Perše and Cerar 2011). In this study, the majority of ACF were developed in the middle and distal colon. Higher risk of developing colon cancers has been related with higher numbers of ACF and higher crypt multiplicity (Orlando



**Fig. 3** **a** Histology of normal colon mucosa of one of the individuals in the control group. It shows the crypts of Lieberkuhn and the smooth muscle; **b** dysplasia of the group treated with DMH, a high

degree of dysplasia is observed with a total loss of the anatomy of the normal mucosa; **c** colon mucosa of a group treated with *C. citrinus* leaves extract. Hematoxylin–Eosin stain ( $\times 40$ )



**Fig. 4** Effects of *Callistemon citrinus* leaves extract on DMH-induced ACF in rat's colon: **a** normal crypts of control group; **b** aberrant crypt foci in carcinogen group; **c** ACF in the *C. citrinus* leaves extract + DMH-treated group. Methylene blue staining (0.2%)  $\times 40$

**Table 2** Effect of *Callistemon citrinus* extract (*C.c*) on DMH-induced ACF and crypt multiplicity in colon of rats after 22 weeks

ACF	Proximal		Middle		Distal	
	DMH	DMH + <i>C.c</i>	DMH	DMH + <i>C.c</i>	DMH	DMH + <i>C.c</i>
2	0	0	0	0	1 ± 0.48	6 ± 0.83*
3	4 ± 1.03*	0	2 ± 0.40	0	3 ± 0.83*	1 ± 0.40
4	0	0	2 ± 0.40	1 ± 0.40	4 ± 0.40	4 ± 0.51
5	4 ± 2.16*	0	2 ± 0.81	2 ± 0.81	6 ± 2.00*	1 ± 0.40
6	0	0	2 ± 0.40	0	3 ± 0.83*	0
7	1 ± 0.40	0	1 ± 0.40	0	1 ± 0.40	0

Aberrant crypt foci (ACF) are categorized by number of crypts per focus: 1–7. Values are mean ± SD (\* $p \leq 0.05$ ,  $n = 6$ , Tukey test)

et al. 2008). Rats fed with *C. citrinus* showed lower crypt multiplicity in the middle and distal colon comparing with DMH-treated group (Table 2).

This result is in agreement with Almagami et al. (2014) who reported a decrease in the number of ACF, after administration of 250 and 500 mg/kg b.w. diary oral dose, of *Acanthos ilicifolius* extract against azoxymethane-induced ACF in the rat colon. A good therapeutic solution to avoid CRC should be use to prevent ACF formation. In this study, the presence of ACF at 22 weeks indicates that the carcinogenic process continues in the DMH-treated group. However, in the *C. citrinus* extract-treated group, the ACF formation and the colonic crypts decreased, and the staining with methylene blue was similar to that of normal crypts, while in the DMH-treated group were darker than the normal crypts (Fig. 4).

The major compounds in leaves of *C. citrinus* are the 1,8-cineole (14.73%), limonene (9.12%), and  $\alpha$ -terpineol (5.41%) (Petronilho et al. 2013). These compounds are recognized as potential chemotherapeutic agents (Murata et al. 2013; Jia et al. 2013; Hassan et al. 2010). Limonene also has been reported to reduce the number of aberrant crypts and multiplicity crypts induced by azoxymethane (Kawamori et al. 1996). In this study, the same effect was observed in the *C. citrinus* extract-treated rats.

### Serum proteins and albumin determinations

Total proteins and albumin were evaluated in serum by colorimetric assays (Table 3). The ANOVA test did not show significant differences between the groups ( $p = 0.34$ ). However, the protein values in the DMH group were the lowest and below the range reported as normal for 5-month-old male rats. Changes in the levels of serum proteins are indicators of colon cancer (Kuppusamy et al. 2014).

The values of serum albumin were similar between the treatments, there was no significant difference ( $p = 0.54$ ), and they were in the range of normal values. Albumin is the most important protein in the human serum. It has been reported that low level of albumin means a metastasis

**Table 3** Total proteins and albumin determination in serum

Serum determinations (mg/mL)	Control	DMH	DMH + extract	Normal value <sup>a</sup>
Total proteins	6.3 ± 0.9	5.4 ± 0.8	6.1 ± 0.3	5.9–7.9
Albumin	2.9 ± 0.2	2.9 ± 0.2	3.1 ± 0.4	2.4–4.6

Values are given as mean ± SD ( $n = 6$ )

<sup>a</sup>Normal values according to León-Goñi et al. (2011)

process (Boonpipattanapong and Chewatanakornkul 2006). In our results, there is no evidence of metastasis process.

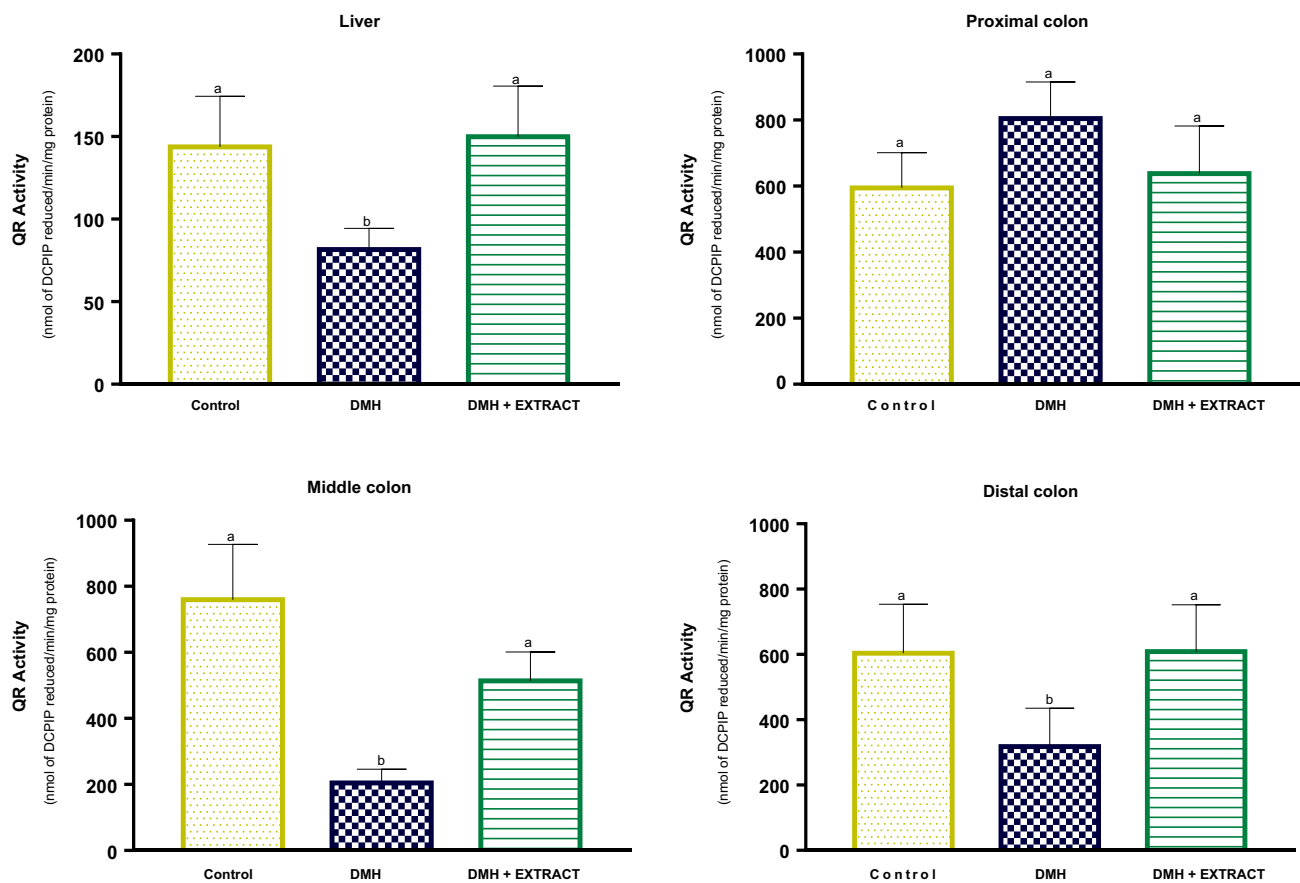
### Phase II enzyme activities

Quinone reductase (QR) is one of the phase II enzyme that plays an important role of detoxification of hazard compound. QR catalyzes two-electron reduction of quinones and quinoid compounds from sources endogenous and exogenous. QR helps the cells to control the production of reactive oxygen species. (Qiang et al. 2004; Talalay and Dinkova-Kostova 2004; Froyen and Steinberg 2011).

Figure 5 displays the activity of quinone reductase. The group treated only with DMH showed a significant decrease (Tukey,  $p < 0.05$ ) with respect to the control group of the QR activity in the liver (40% less), the middle colon (71% less) and the distal colon (43% less). The decrease in the activity of this enzyme corresponds to those areas where the major number of tumors and ACF were recorded.

Our results showed that the QR activity in the liver was similar to the control group in the *C. citrinus* extract-treated group, meanwhile the activity of QR decreased in the liver of DMH-treated group.

In the proximal colon, QR activity did not show any difference between treatments. This colon segment presented smaller number and size of tumors and the presence of ACF were lower than the observed on other two fragments. In the middle colon, the group administered with the extract showed a decrease in the activity of the enzyme compared to



**Fig. 5** Quinone reductase (QR) activity in the different tissues of three experimental groups. Different letters indicate significant differences between treatments of the same tissue (Dunnett  $p \leq 0.05$ ,  $n = 6$  (liver) and  $n = 6$  (colon)  $\pm$  SD)

the control, but significantly higher than that DMH-treated group. Finally, in the distal zone, the QR activity decreased significantly on exposure to DMH, this adverse effect was reversed to normal values on the *C. citrinus*-treated group with the carcinogen.

The fact that QR activity between the control group and the *C. citrinus*-treated group was similar suggests the potential value of this plant as a protective agent against chemical carcinogenesis.

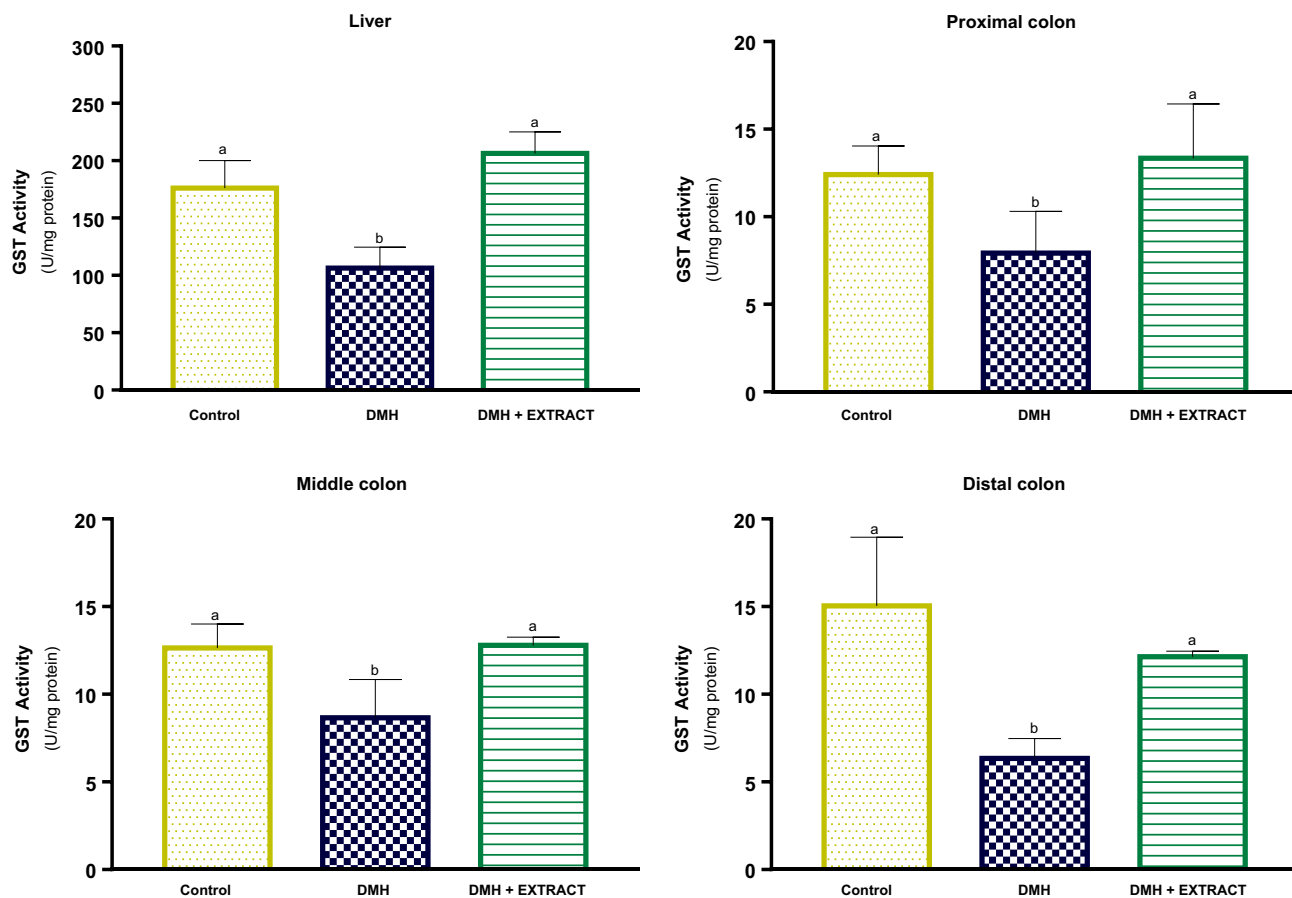
The decrease in the number of animals with tumors and precancerous lesions (ACF) in the groups treated with the extract supports the previous statement that induction of QR activity assists in chemoprevention against cancer and chemical toxicity by natural or synthetic compounds (Cuenet et al. 2006).

The glutathione *S*-transferases (GSTs) is another detoxification enzymes of phase II used as nucleophilic one molecule of reduced glutathione (GSH). It attacks compounds that contain electrophilic centers and produces glutathione-conjugates (Sherratt and Hayes 2002). Figure 6 presents the activity of GST in the liver and the three portions of colon in control and experimental rats.

The activity of GST was significantly decreased in the liver and in the three segment of the colon: proximal, middle and distal of DMH alone treated group as compared to the control group; meanwhile the activity of GST in *C. citrinus* extract plus DMH-treated group was similar as the control group in the liver and in the three segment of the colon: proximal, middle and distal.

The present study shows significant decrease of the activities of QR and GST on exposure to DMH, whereas the addition of *C. citrinus* extract to DMH-treated rats reversed their levels. This result suggests that the *C. citrinus* induced the activity of the phase II enzymes to avoid the deleterious effect of the DMH.

Our results are in agreement with those obtained by Piña (2007) who evaluated the phase II enzyme-inducing potential of the extracts of *Portulaca oleracea* from Mexico in a model of carcinogenesis with DMH associated with inflammation. The results obtained showed that the intragastric administration of aqueous and ethanolic extracts are able to induce the activity of QR and GST at different doses obtaining the highest induction with the aqueous extract at a dose of 100 mg/kg.



**Fig. 6** Glutathione *S*-transferase (GST) activity in the different tissues of three experimental groups. Different letters indicate significant differences between treatments of the same tissue (Dunnett  $p \leq 0.05$ ,  $n = 6$  (liver) and  $n = 6$  (colon)  $\pm$  SD)

Many natural products have been studied in models of colon cancer; for example, garlic has chemoprotective effects against CRC. It contains several compounds that inhibit carcinogen activation, mutagenesis, enhancement of detoxification, and protects DNA from activated carcinogens (Sporn et al. 1988; Katsuki et al. 2006). Curcumin has anti-inflammatory activity (Jurenka 2009), inhibiting the cytochrome P-450 enzyme activity and increasing the levels of glutathione *S*-transferase (Chauhan 2002); green tea prevents tumor invasion and angiogenesis (Du et al. 2012) and it has anti-inflammatory effect (Peng et al. 2006). Farnesol, an organic natural compound from many plants, effectively suppresses DMH-induced colonic mucosal damage by ameliorating oxidative stress, inflammatory and apoptotic responses (Khan and Sultana 2011).

The induction of phase II enzyme NAD(P)H:quinone reductase (QR) and GST is one of the principal mechanisms of protection against drug toxicity and carcinogenesis (Cuendet et al. 2006). Ansil et al. (2013) found a chemopreventive effect of *Amorphophallus campanulatus* (Roxb) through the increase of GST activity in colon cancer

in Wistar rats. Reynoso-Camacho et al. (2015) showed that consumption of tortillas specially made of white and blue corns induce the activity of QR and GST against colon cancer in Sprague–Dawley rats. Tan and Spivack (2009) reported that the intake of higher fiber found in the fruits and vegetables induce the expression of phase II enzymes and protect against lung cancer. Limonene showed anticarcinogenic activity by the induction of GST during mammary carcinogenesis (Elegbede et al. 1993) and hepatocarcinogenesis (Parija and Das 2003). Limonene is the second major compound in *C. citrinus*.

Conversely 1,8-cineole, limonene and  $\alpha$ -terpineol present in *C. citrinus* extracts might enhance the detoxification mechanism by modulating phase II enzyme activity. This effect could be associated with the inhibition of cell proliferation and induction of tumor cell death. In this context, previous studies have shown that *C. citrinus* and some compounds possess anti-inflammatory, antioxidant and cytotoxic properties, which could contribute to its chemopreventive activity (Murata et al. 2013; Jia et al. 2013; Hassan et al. 2010).



This study presents the first evidence of a chemopreventive effect of *Callistemon citrinus* via enzymatic induction in in vivo models.

## Conclusion

Our results suggest that oral administration of *Callistemon citrinus* leaves extract decreases the number of ACF, crypt multiplicity and the tumor sizes in animals with colorectal cancer. Moreover, 33% of the rats did not present any ACF or tumor. Additionally, *Callistemon citrinus* has a chemopreventive effect by increasing the activity of phase II detoxification enzymes, quinone reductase and glutathione *S*-transferase. These findings propose that *C. citrinus* could be a potential chemopreventive agent against colon rectal cancer.

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**Author contributions** PR-C was involved in the manuscript preparation, designed and supervised this research. Students AL-M and LGOP performed the laboratory tests. DG-H and BN-M supervised animals' experimentation and EM-H supervised the histological analysis. All the authors approved the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The experimental protocol and animal procedures were approved by the Institutional Bioethics and Biosecurity Committee in the Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolas de Hidalgo (approval date: 01/02/2017; Protocol ID IIQB-CIBE-05-2017). This research accomplishes with the Mexican Regulations (NOM-062-ZOO-1999, SAGARPA, Mexico) of technical specifications for production, management, care and use of laboratory animals.

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