ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Technol. Aliment. 21(1) 2022, 101–109

www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://dx.doi.org/10.17306/J.AFS.2022.0998

ORIGINAL PAPER

Received: 29.08.2021
Accepted: 3.01.2022

ANTI-OBESITY, SLIMMING, BIOCHEMICAL AND GENOTOXIC EFFECTS OF *CORDIA ECALYCULATA* IN DIET-INDUCED OBESE RATS

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ABSTRACT

Background. Overweight and obesity are associated with deaths and diseases worldwide. *Cordia ecalyculata* is a plant marketed as a slimmer.
Methods. The study evaluated the anti-obesity effects of the dry extract from *C. ecalyculata* in rats fed with a standard diet (STD) or cafeteria diet (CD) receiving the dry extract from *C. ecalyculata* at 500, 1000, and 2000 mg/kg for 40 days. Furthermore, it evaluated the slimming effect on diet-induced obese rats by the treatment with the same doses for 30 days. The bodyweight of the rats, as well as the intake of food, was measured. Blood samples were collected to determine the liver function (albumin, alanine transaminase (ALT), alkaline phosphatase (ALP), glucose), renal function (urea and creatinine), and lipid profile (cholesterol, triglycerides).
Results. The genotoxic effect in peripheral blood was assessed through the comet assay. A lower *C. ecalyculata* dose significantly prevented the weight gain in rats fed with STD and CD and decreased body weight and intake food of obese rats. The biochemical parameters were not altered, except to increase the serum albumin. Only the higher dose induced DNA damage when evaluated in rats fed with CD in the slimming evaluation model used.
Conclusion. These results reinforce the extract as an anti-obesity and slimming supplement.

Keywords: comet assay, *Cordia ecalyculata*, hepatic profile, obesity

INTRODUCTION

According to the World Health Organization (WHO, 2020), overweight and obesity are linked to deaths worldwide. Furthermore, overweight and obesity, as well as their related noncommunicable diseases, are largely preventable. Herbal use is associated with health promotion (Wang et al., 2014), therefore many people use herbal medicines for weigh loss (Yun, 2010). This fact makes this kind of supplements very popular and commonly used worldwide since it leads to less or no side effects when compared to traditional pharmacological treatments (Hasani-Ranjbar et al., 2013).

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The main substances found in plants of the Boraginaceae family are alkaloids, quinones, naphthoquinones, saponins, tannins, phenolic acids, allantoins, mucilages, polysaccharides, flavonoids, cyclitols, and fatty acids of therapeutic and nutritional interest, such as gamma-linolenic acid (Velasco and Goffman, 1999). In this sense, the extract from the Brazilian plant belonging to the Boraginaceae family *Cordia ecalyculata* Vell. (synonym *C. salicifolia* Cham.), popularly known as "porangaba", "buggy tea", or "coffee bush" shows diuretic activity, leading to appetite suppression and weight reduction (Araldi et al., 2014; Menghini et al., 2008), as well as hypolipidemic effects (Siqueira et al., 2006). Phytochemical investigation on leaf extract of *C. ecalyculata* demonstrates high concentrations of caffeine, potassium, allantoic acid, and allantoin (Menghini et al., 2008). Furthermore, according to Volp et al. (2008), *C. ecalyculata* is rich in tannins and anthocyanins, which have antioxidant properties that can modulate the expression of adipokines and prevent fat accumulation.

Given that *C. ecalyculata* has been ethnopharmacologically referenced as an alternative treatment for obesity and has the pharmacological potential for the development of phytotherapeutic medicines, this study aimed to evaluate the anti-obesity and slimming effects in diet-induced obese rats. In addition, liver and kidney biochemical parameters were evaluated, as well as the genotoxic effects in the peripheral blood of animals treated sub chronically with the extract of *C. ecalyculata*.

METHODS

Animals

Ninety-six Wistar albino male rats aged 8–10 weeks old and weighing around 200–250 g from the Vivarium of Lutheran University of Brazil (ULBRA) were housed four per cage under 12–12 h daylight cycle and 23 ±2°C temperature. The experimental protocol was approved by the Ethics Committee on Animal Use at ULBRA (under protocol 2011-42P) and animal care was taken according to the guidelines as indicated in the recommendations of the National Institutes of Health Guide for the care and use of laboratory animals.

Collection of plant material

The dry extract of the leaves and stems of *C. ecalyculata* in powder form obtained from maceration was purchased from a raw material distributor in the state of Rio Grande do Sul (Embrafarma), Brazil, without additives. The test substance was presented in a 500 g package, and closed with a certification seal and a quality guarantee. The phytochemical analysis label, evaluated by the manufacturer, showed the presence of caffeine, allantoin, and tannins present.

Experimental protocols

Firstly, the animals were divided into two groups: Standard diet (STD) or Cafeteria diet (CD). Cafeteria diet was chosen because according to Buyukdere et al. (2019), it showed more pronounced obesity when compared to a high-fat diet in young male rats. In the current study, the STD was composed of the dry pellet Nuvilab CR® (Quimtia, Paraná, Brazil) containing crude protein (22 to 22.5%), lipids (4.4%), and carbohydrates (53 to 55%), totaling 295 kcal/100 g. The proposed CD includes supplementation with hypercaloric foods, and the average energy composition was 9% protein, 31.5% carbohydrates, and 60% lipids, adding 340 kcal in 100 g. Cafeteria diet was prepared using (Jindal et al., 2011): a – bread (25 g) + condensed milk (25 g), b – chocolate biscuits (25 g) + potato chips (25 g), c – potato chips (25 g) + rice polish (25 g). These supplementations were offered along with STD for 3 days, in a sequence of a, b, and c in rotation, for a total period of 30 days or 70 days, depending on the evaluation model. The animals received water *ad libitum*.

For the experiment, the test substance was homogenized in saline plus 0.05% carboxymethylcellulose and administered orally (via gavage) to the animals. Control animals received only carboxymethylcellulose (0.05%). The doses used in this study were based on the results of Caparroz-Assef et al. (2008) and Dias (2004) who showed no mortality and acute toxicity of the hydroethanolic extract of the leaves of *C. ecalyculata* (5000 mg/kg) when it was administered for 15 days in male and female Wistar rats. Thus, the chosen doses were 500 mg/kg, 1000 mg/kg, and 2000 mg/kg. To evaluate the effect of *C. ecalyculata* as an anti-obesity supplement, the animals were fed for 40 days with STD and CD together with the daily administration of the test substance in three different doses. To evaluate the effect on slimming, after 40 days from the beginning of the STD or CD feeding, the animals then maintained their diet for another 30 days plus daily treatment with the dry extract from *C. ecalyculata*

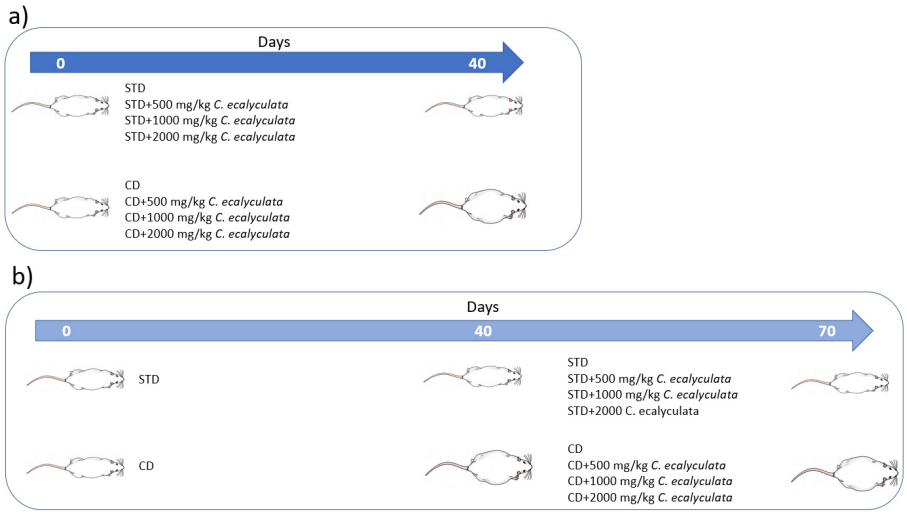

Fig. 1. Experimental design. Evaluation of dry extract of *C. ecalyculata* as anti-obesity (**a**) and slimming (**b**): STD – standard diet, CD – cafeteria diet. Each group was formed by 6 animals ($n = 6$)

in the same three different dosages (Fig. 1). The animals were weighed at the beginning and end of the study. Each experimental group was formed by 6 animals ($n = 6$) and physical activity status was not assessed.

Measurement of food intake

The food intake of each animal was determined initially and then every week thereafter by measuring the difference between the pre-weighed pellets and the weight of the food that remained after 24 hours.

Biochemical parameters

At the end of the experimental protocols, the rats were euthanized by an isoflurane overdose. Blood samples were collected in a Vacutainer® tube with EDTA to determine the liver function (albumin, alanine transaminase (ALT), alkaline phosphatase (ALP), glucose), renal function (urea and creatinine), and lipid profile (cholesterol, triglycerides).

Genotoxicity assay

Peripheral blood was used to perform the alkaline comet assay, as described by Tice et al. (2000), with minor modifications. As a positive control hydrogen peroxide (0.25 mM solution) was applied to slides for 5 min, at a 4°C temperature. The extent of DNA damage was determined by classifying comets into five categories, based on the length of DNA migration and/or of the perceived relative proportion of the DNA in the tail to the size of the head (nucleus). The categories were zero, which represented undamaged cells (comets with no tail), and categories 1–4 that represented increasing relative tail intensities and smaller head sizes. The parameter used to evaluate DNA damage was the damage index (DI) and damage frequency (DF). The DI of a group could range from zero (completely undamaged = 100 cells × 0) to 400 (maximum damage = 100 cells × 4). The DF was calculated based on the number of cells with a tail versus those without a tail.

Statistical analyses

The data were expressed as mean ±standard deviation. The statistical analyses were carried out by a One-Way Analysis of Variance (ANOVA), followed by the post hoc test or Kruskal Wallis test (GraphPad Prism 5.0, USA). In all comparisons, $p \leq 0.05$ was considered an indicator of statistical significance.

RESULTS

Anti-obesity effects of *C. ecalyculata*

Animals that received STD or CD plus *C. ecalyculata* 500 mg/kg for 40 days, showed a significant decrease in weight gain. Curiously, the higher dose did not show significant effects. Furthermore, the intermediate dose in the animals fed with STD and treated

Table 1. Anti-obesity effects of dry extract of *C. ecalyculata* p.o. for 40 days in the bodyweight of animals under standard diet (STD) or cafeteria diet (CD)

Group	Mean ±SD, %
STD	29.9 ±8.7
STD 500 mg/kg *C. ecalyculata*	11.1 ±6.6*
STD 2000 mg/kg *C. ecalyculata*	25.9 ±5.9
CD	50.9 ±9.7
CD 500 mg/kg *C. ecalyculata*	24.1 ±4.2**
CD 1000 mg/kg *C. ecalyculata*	39.0 ±6.8
CD 2000 mg/kg *C. ecalyculata*	36.6 ±6.8

$n = 6$ animals per group.
*$p < 0.001$ when compared to the STD group. **$p < 0.001$ when compared to the CD group.

Table 2. Slimming effects of dry extract of *C. ecalyculata* p.o. for 30 days in the bodyweight of animals under standard diet (STD) or diet-induced obese rats

Group	Mean ±SD, %
STD	6.5 ±5.1
STD 500 mg/kg *C. ecalyculata*	–4.7 ±5.6*
STD 1000 mg/kg *C. ecalyculata*	2.5 ±4.3
STD 2000 mg/kg *C. ecalyculata*	3.7 ±14.0
CD	4.6 ±1.4
CD 500 mg/kg *C. ecalyculata*	–7.3 ±6.3**
CD 1000 mg/kg *C. ecalyculata*	–1.9 ±4.5***
CD 2000 mg/kg *C. ecalyculata*	–2.0 ±4.7***

$n = 6$ animals per group. CD – cafeteria diet.
Negative results mean a decrease in the body weight of the animals. *$p < 0.001$ compared to the STD group. **$p < 0.001$ and ***$p < 0.05$ compared to the CD group.

with *C. ecalyculata* 1000 mg/kg presented a high SD, becoming an inconclusive result (data not shown). Related to animals fed with CD, the lower dose also was significantly effective in preventing weight gain (Table 1).

Slimming effects of *C. ecalyculata*

A significant decrease in weight after the treatment with *C. ecalyculata* 500 mg/kg for 30 days was observed in rats fed with STD. However, in animals fed with CD, the loss of weight was observed in all groups treated with *C. ecalyculata*. The effect in rats fed with STD and treated with *C. ecalyculata* 500 mg/kg for 30 days was significant compared to other doses. On CD-animals, it was possible to observe the same pattern of effect, although the intermediate and higher doses showed smaller significant effects (Table 2).

Effect of *C. ecalyculata* on calorie intake

The *C. ecalyculata* at 500 mg/kg treatment was able to significantly reduce the intake calories of rats under STD and CD in the anti-obesity evaluation. Furthermore, the same result can be observed in relation to the slimming evaluation (Table 3).

Table 3. Effects of *C. ecalyculata* p.o. for 40 days in rats under standard diet (STD) or cafeteria diet (CD) (anti-obesity) and 30 days in obese animals under STD or CD (slimming) in the calories intake

Group	Anti-obesity	Slimming
STD	2 709 ±94.9	2 492 ±38.6
STD 500 mg/kg *C. ecalyculata*	2 110 ±94.6*	2 078 ±139.7*
STD 1000 mg/kg *C. ecalyculata*	2 039 ±156.2*	2 409 ±88.1
STD 2000 mg/kg *C. ecalyculata*	2 256 ±142.0	2 223 ±85.27
CD	2 854 ±79.4	2 734 ±133.2
CD 500 mg/kg *C. ecalyculata*	2 284 ±165.7***	2 031 ±53.55**
CD 1000 mg/kg *C. ecalyculata*	2 419 ±148.6	2 120 ±90.7***
CD 2000 mg/kg *C. ecalyculata*	2 318 ±140.8	2 243 ±176.1

$n = 6$ animals per group.
Data expressed as mean ±SD of energy intake for a group of six rats in kcal/week. *$p < 0.05$ when compared to the STD group and **$p < 0.001$. ***$p < 0.05$ when compared to the CD group.

Biochemical parameters

Among the biochemical parameters evaluated, this study showed that albumin was significantly elevated

in groups fed with STD for 40 days and daily treated with *C. ecalyculata* 500 mg/kg ($p < 0.05$), 1000 and 2000 mg/kg ($p < 0.001$) for another 30 days (Table 4). Furthermore, the rats receiving CD and treated with 1000 and 2000 mg/kg of *C. ecalyculata* also showed albumin and glucose increases, although insignificant values.

The other biochemical parameters evaluated from the *C. ecalyculata* treated groups did not demonstrate any significant values compared to their respective control groups, except for a significant increase of ALP in an anti-obesity group fed with STD and treated with 1000 mg/kg (Table 4). On the other hand, when the obese-induced animals were treated with *C. ecalyculata*, only the group treated with a 1000 mg/kg dose showed a significant increase in ALP.

Genotoxic effects

Regarding anti-obesity evaluation, DI and DF values in groups treated with *C. ecalyculata* were similar compared to the respective control groups, except for a significantly decreased DF value observed in STD 500 mg/kg. However, there was an increase in DI and DF values in the group fed with CD and treated with 2000 mg/kg for 30 days, suggesting induction of DNA damage in the highest dose (Table 5).

DISCUSSION

C. ecalyculata is marketed as an appetite reducer (Alexandre et al., 2018). According to Assonuma (2009), this plant contains phytochemical constituents of alkaloids like caffeine, allantoins, and allantoic acid,

Table 4. Biochemical effects of dry extract of *C. ecalyculata* p.o. for 40 days in rats under standard diet or cafeteria diet (anti-obesity) and 30 days in animals under standard diet or diet-induced obese rats (slimming)

Parameter	Evaluation	STD	STD 500 mg/kg *C. ecalyculata*	STD 1000 mg/kg *C. ecalyculata*	STD 2000 mg/kg *C. ecalyculata*	CD	CD 500 mg/kg *C. ecalyculata*	CD 1000 mg/kg *C. ecalyculata*	CD 2000 mg/kg *C. ecalyculata*
Albumin	anti-obesity	1.9 ±0.4	2.7 ±0.9	2.4 ±0.8	1.9 ±0.3	2.4 ±0.6	2.0 ±0.5	2.0 ±0.5	2.5 ±0.8
	slimming	2.1 ±0.5	3.5 ±0.4*	4.0 ±0.3**	3.2 ±0.7**	2.9 ±0.6	4.2 ±0.9	4.7 ±0.6***	6.0 ±0.9***
ALT	anti-obesity	30.4 ±10.0	33.6 ±5.2	52.5 ±13.3	34.4 ±12.7	43.2 ±16.9	35.3 ±8.2	49.1 ±26.7	33.9 ±10.2
	slimming	42.5 ±5.4	42.6 ±8.9	40.0 ±3.9	43.8 ±1.6	44.7 ±6.8	34.0 ±8.1	28.7 ±9.8	38.8 ±10.1
ALP	anti-obesity	94.7 ±14.7	124.6 ±45.5	220.2 ±46.5**	108.3 ±48.1	111.7 ±21.5	81.5 ±20.7	145.7 ±31.6	134.7 ±30.8
	slimming	110.8 ±22.2	116.1 ±20.3	83.8 ±45.9	148.9 ±61.2	106.7 ±21.7	121.4 ±28.3	191.2 ±150.5	132.6 ±53.6
Glucose	anti-obesity	58.8 ±20.9	75.8 ±9.0	63.4 ±16.1	61.6 ±17.0	66.7 ±16.7	82.8 ±17.1	87.7 ±27.2	71.7 ±34.5
	slimming	140.6 ±28.8	134.6 ±34.6	129.5 ±29.4	121.9 ±26.7	163.7 ±7.8	163.8 ±35.1	210.7 ±28.8	179.1 ±28.9
Urea	anti-obesity	43.2 ±3.8	46.5 ±8.6	42.3 ±5.5	41.6 ±8.7	38.9 ±2.4	40.3 ±5.3	39.1 ±3.3	49.5 ±12.5
	slimming	55.4 ±9.5	51.0 ±8.9	54.2 ±17.0	74.3 ±15.2	44.2 ±2.7	50.1 ±8.1	52.1 ±7.9	59.4 ±5.6
Creatinine	anti-obesity	0.6 ±0	0.6 ±0.1	0.6 ±0	0.5 ±0.1	0.5 ±0.1	0.6 ±0.2	0.6 ±0.1	0.7 ±0.2
	slimming	0.7 ±0.1	0.9 ±0.2	0.7 ±0.2	0.8 ±0.2	0.7 ±0.3	0.8 ±0.1	0.8 ±0.1	0.6 ±0.1
Total cholesterol	anti-obesity	97.4 ±57.8	104.6 ±27.4	157.1 ±41.4	89 ±28.7	67.2 ±15.1	87.8 ±18.2	77.2 ±20.4	135.9 ±48.3
	slimming	86.6 ±8.2	116.3 ±21.5	101.5 ±12.8	92.5 ±24.0	98.3 ±22.5	114.6 ±18.9	96.3 ±18.0	129.9 ±35.3
Triglycerides	anti-obesity	64.9 ±52.0	70.0 ±42.5	52.7 ±44.6	37.1 ±10.5	44.4 ±12.0	116.5 ±20.3	80.1 ±29.9	49.4 ±17.2
	slimming	50.7 ±10.5	70.5 ±27.0	64.6 ±15.9	59.6 ±22.0	92.5 ±52.7	52.3 ±11.6	60.0 ±12.5	79.8 ±16.7

$n = 6$ animals per group. STD – standard diet, CD – cafeteria diet. Data expressed as mean ±SD.
$^{*}p < 0.05$ and $^{**}p < 0.01$ when compared to the STD group. $^{***}p < 0.01$ when compared to the CD group.

Table 5. Genotoxic effects of *C. ecalyculata* p.o. for 40 days in rats under standard diet or cafeteria diet (anti-obesity) and 30 days in animals under standard diet or diet-induced obese rats (slimming)

Evaluation	Evaluation	STD	STD 500 mg/kg *C. ecalyculata*	STD 1000 mg/kg *C. ecalyculata*	STD 2000 mg/kg *C. ecalyculata*	CD	CD 500 mg/kg *C. ecalyculata*	CD 1000 mg/kg *C. ecalyculata*	CD 2000 mg/kg *C. ecalyculata*
Anti-obesity	DI	56.8 ±25.9	43.0 ±8.2	84.8 ±37.5	84.6 ±51.8	53.6 ±35.9	60.0 ±28.4	35.8 ±4.6	41.0 ±18.2
	DF	28.4 ±7.3	18.2 ±3.6*	39.2 ±20.1	39.0 ±16.1	24.2 ±15.9	36.2 ±15.1	17.6 ±3.4	21.0 ±11.7
Slimming	DI	32.8 ±25.5	25.4 ±7.9	27.8 ±24.9	17.6 ±7.1	13.2 ±4.4	15.4 ±8.7	26.2 ±18.5	91.6 ±32.3**
	DF	23.0 ±14.6	13.4 ±3.9	12.0 ±7.4	7.4 ±1.8	12.0 ±3.1	8.4 ±4.1	15.0 ±7.9	57.2 ±17.3**

$n = 6$ animals per group. STD – standard diet, CD – cafeteria diet, DI – damage index, DF – damage frequency.
Data expressed as mean ±SD, $^{*}p < 0.05$ when compared to the STD group. $^{**}p < 0.01$ when compared to the CD group.

glycosides, tannins, and flavonoids. In this sense, extracts from plants showing those compounds have been proven to regulate adipogenesis (Wang et al., 2020). No data mentions the usual doses in the clinical field, therefore, no scientific basis is given to justify the suggested doses in the drugstores which market the extract (125–300 mg twice a day) (Pelizza, 2010). Due to the scarcity of scientific evidence on therapeutic effects of the dry extract of *C. ecalyculata*, this study evaluated the anti-obesity and slimming effects in rats fed with STD or CD and treated orally with the extract, since this plant is administered using this via in human. Even so, it is needed to point out that the exact mechanism of action is unknown, as well as whether it can be directly transferred to humans.

The lower dose (500 mg/kg) of *C. ecalyculata* administrated for 40 days significantly prevented the weight gain in rats being fed with STD or CD. Furthermore, using the same dosage administered for 30 days, rats fed with STD and rats fed with CD became thin. Conversely, in previous studies by Santos (2014) and Colli et al. (2016), a hydroethanolic extract of *C. ecalyculata* at 20, 100, and 400 mg/kg for 60 consecutive days orally did not show a reduction in body weight. In addition, Caparroz-Assef et al. (2008) have shown that the daily oral administration of a *C. ecalyculata* aqueous extract at 20, 100, 200, and 400 mg/kg for 90 days did not cause changes in body weight gain. Also, da Silva et al. (2010) have observed that the oral treatment with the crude extract from *C. ecalyculata* 2000 mg/kg for 15 days did not reduce body weight gain or the amount of food consumed by Swiss mice. Siqueira et al. (2006) investigated the dried, powdered leaves of *C. ecalyculata* (20 mg/kg) administered by gavage for 13 days and did not find anti-obesity, appetite suppressant, and diuretic effects, but the hypolipidemic effect in normal and alloxan-diabetic rats was observed. However, corroborating in part with the currrent results, Araldi et al. (2014) found weight reduction in female Swiss mice in groups receiving 150 and 300 mg/kg *C. ecalyculata* orally for 7 consecutive days, receiving a standard diet.

In this study, the biochemical parameters evaluated were not altered, except for the serum albumin which showed an increase when both the anti-obesity and slimming evaluations were performed, mainly in rats fed with STD and CD receiving *C. ecalyculata* at 1000 and 2000 mg/kg. The serum albumin levels increase is linked to hepatic injury, and it is commonly an increase in the serum ALT and AST levels (Brisotti et al., 2000; Hamid et al., 2012; Jackson et al., 2008). When the anti-obesity effects were evaluated in rats fed with STD treated with 1000 mg/kg, the serum ALP levels were increased. According to Xu et al. (2002), little is known about the role of ALP in liver diseases. However, the ALP results found here are not able to suggest hepatotoxicity caused by the dry extract of *C. ecalyculata*, and further studies to measure other biomarkers and analyzing the liver histopathology are needed. Cardozo et al. (2008) found a reduction in serum total cholesterol in mice fed with STD and treated orally with a *C. ecalyculata* dry extract (100 mg/kg for 15 days), as well as a reduction in the serum triglyceride in animals under hyperlipidemia diet plus extract. Here, total cholesterol and triglyceride values remained at normal levels in all groups studied,

indicating that the extract was not able to alter the lipid profile. Therefore, looking at the biochemistry panels, there is no dyslipidemia present and no obese animals showed alterations in glucose levels, showing a little evidence that the rats had obesity that could be alleviated in the slimming study.

Caparroz-Assef et al. (2008) revealed that the daily oral administration of an aqueous extract (20, 100, 200, and 400 mg/kg) for 90 days did not cause changes in organ weight, hematological, and biochemical parameters of the animals, that is, it did not cause toxicity under chronic use. However, the reproductive toxic potential of different doses of the extract of *C. ecalyculata* in adult rats showed that the weight of the testicles, seminal glands, and prostate were not affected by treatments, but there was an increase in the epididymis weight at a dose of 400 mg/kg of the extract administered for 60 consecutive days. Besides, at all doses, the testicles and epididymis showed histopathological changes with an increase in the percentage of abnormal gametes and a decrease in fertility of animals (Colli et al., 2016; Santos, 2014).

Regarding genotoxic evaluation using comet assay, only the higher dose (2000 mg/kg) was able to increase DNA damage after 30 days of administration in rats fed with CD (Table 4). Interestingly, the extract did not show genotoxic effects in rats fed with STD, suggesting an influence of the diet on these results. It is known that some diets might disturb genomic stability mainly by altering the cellular redox state (Minihane et al., 2015; Włodarczyk and Nowicka, 2019). Thus, the extract induced DNA damage in rats fed with CD, but not in rats fed with STD, likely because CD impaired DNA repair, decreasing the genomic stability and favoring the induction of DNA damage by chemical substances in the extract. Da Silva et al. (2010) have shown that a *C. ecalyculata* crude extract at 500, 1000, and 2000 mg/kg did not increase DNA damage in blood and micronucleus frequency in bone marrow polychromatic erythrocytes of male Swiss mice, 24 h after the treatment. Also, Araldi et al. (2014) did not observe clastogenic activity in bone marrow polychromatic erythrocytes of mice treated for 7 consecutive days in dose up to 500 mg/kg, suggesting that the extract did not induce genotoxic and mutagenic activities. In those studies, a standard diet was used, corroborating the lack of genotoxic effects of the extract in rats fed with SDT. In the anti-obesity evaluation, the extract was not able to induce DNA damage, even in rats fed with CD. In this case, the extract probably avoided the increase of genomic instability that could be caused by the CD, since the treatments started simultaneously with the diets.

Taken together, the results demonstrated that the lower dose of the dry extract of *C. ecalyculata* has anti-obesity and slimming effects in both STD and CD groups, without inducing DNA damage or altering biochemical parameters (Fig. 2). The reduction in the

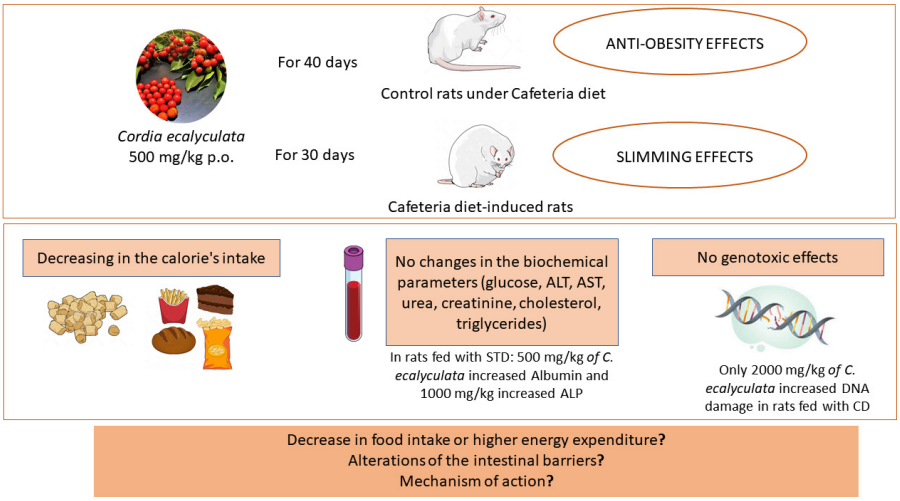

Fig. 2. Graphical conclusion

final body weight could be due to a decrease in food intake or higher energy expenditure (Perumal et al., 2021), and it is in line with the present findings. Moreover, alterations of the intestinal barriers also could cause the inhibition of body weight gain (Karimi et al., 2015). These results indicate this plant is promising as an anti-obesity and slimming supplement, although more studies are needed to assess its safety and efficacy. Elucidation of its mechanism of action is also needed, which could be derivative from the phytochemical components of leaf extract of *C. ecalyculata.*

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