Original Article

# Impact of *Cassia acutifolia* Infusion on Glucose Levels in Obesity and Diabetes Rat Model

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## Abstract

**Objectives:** The aim of this study was to investigate the effects of *Cassia acutifolia* on the obesity and the glucose levels in a rat model of obesity and diabetes.

**Methods:** By random selection, 36 Wistar male rats were divided in two control groups, the positive and the negative control groups, and into four experimental groups receiving different infusions of *Cassia acutifolia* in water *ad libitum*.

**Results:** The results revealed a statistically significant anti-obesogenic effect ($P = 0.02$), although this was not considered clinically significant. Additionally, *Cassia acutifolia* lowered the glucose levels by 30 mg/dL to 90 mg/dL ($P = 0.05$). However, we observed adverse effects in the liver, a two-fold increase in transaminase levels ($P = 0.002$), and in the kidneys, decreased creatinine levels ($P = 0.001$), and these adverse effects had no viable explanation.

**Conclusion:** *Cassia acutifolia* has anti-hyperglycemic effects in obese diabetic rats. However, *Cassia acutifolia* also has adverse effects, so it should not be adminis-

tered to patients.

## 1. Introduction

According to the World Health Organization (WHO), chronic non-communicable diseases are the most common cause of morbidity and mortality worldwide [1]. According to ENSANUT (Nutritional and Health National Test), 9.17% of adult Mexicans, equivalent to 6.4 million people, have reported a previous diagnosis of diabetes by a doctor [2]. Thus, identifying new strategies for treating patients with diabetes is vitally important.

Type 2 diabetes mellitus (DM2) can largely be prevented and treated with changes in diet and lifestyle [3]. However, in the population of Mexico, this type of treatment has not yielded the expected results. When prevention and non-pharmacological treatments are insufficient, pharmacological treatments are required. Most treatments have good results when prescribed correctly based on disease timing, dosage, and patient needs. However, these factors make treatment complicated. Thus, a high percentage of patients do not reach the desired glucose control (i.e., glucose levels ≤ 100 mg/dL) in a reasonable amount of time, which causes many patients to deviate from this type of treatment and seek alternative treatments [3].

In Mexico, alternative treatments are common, especially so-called "remedies". These remedies are often xenobiotics being used to treat or prevent a disease.

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Some of these remedies, for example, nutraceuticals, have motivated increased research because they have had good results. However, a number of these remedies have only anecdotal evidence supporting their efficacy. Furethermore, the effects of some of these remedies may be attributed to the placebo effect, and some remedies may even have adverse effects.

An infusion of the leaves from "senos de mujer," which translates as female breasts in English and which is often referred to as *Senna* or *Cassia acutifolia*, is commonly used in Mexico to treat patients with DM2. The plant is endemic to Africa and Asia, but can now be found throughout Mexico. Traditional thinking and even some professionals suggest drinking an infusion during the day to maintain glucose levels. Even though no studies have been done to verify this claim, anecdotal evidence suggests that consuming the plant has good results. The aim of this study was to evaluate the impact of *Cassia acutifolia* infusion on glucose levels in a rat model of obesity and diabetes.

## 2. Materials and Methods

Male Wistar rats were obtained from the animal house at the University Center Health Sciences, University of Guadalajara. The animals were acclimatized for at least two weeks. The weight of the rats were 100 ± 20 g. They had not previously received any treatments and were without genetic changes. The rats were housed individually and maintained in a well-ventilated animal room (25 - 27°C) with a 12-h light/dark cycle. The rats had free access to a modified diet (high in calories and grass rat chow) and water or modified water (infusion) *ad libitum*.

The animals were handled humanely in accordance with the institutional guidelines of the Ethics Committee of University of Guadalajara, Mexican Official Standard (NOM-062-ZOO-1999), and international guidelines on the Use and Handling of Experimental Animals.

Obesity and DM2 were induced in the rats by feeding them a hypercaloric nutritional diet. The diet was generated from "Purine, Rodent Chow®" that was immersed for 10 minutes in lard that had previously been melted at 90°C and then left at room temperature so that it could be manipulated. Enough sucrose (10% dry weight) was added to cover the food completely and have a homogeneous mixture. Food was offered *ad libitum*.

The model was generated after 10 weeks of feeding, at which time the rats had reached a higher total body weight relative to the healthy controls (> 300 g) [4]. The rats that had been fed a balanced diet (rodent Chow®) reached average weights of 180 - 220 g and were used as negative controls. The dietary modifications are presented in Table 1. Before, during, and after treatment, the body weights of the rats were measured with a special rat scale.

In the model used in this study, DM2 was defined as hyperglycemia, as reported in previous studies, and as a diagnosis of polydipsia, polyuria and polyphagia. Food intake in grams and water consumption in milliliters were recorded. Additionally, urine was collected, and the volume was recorded in milliliters. This served as a control and for comparison following treatment and was used to

**Table 1** Comparisson of Nutritional Information, the food used was Rodent Chow Diet, Purina®

| Diet | Standar | Hypercaloric |
|---|---|---|
| Caloric Density | 3.1 kcal/g | 5.9 kcal/g |
| Proteins | 18% | 14% |
| Lipids | 24% | 38% |
| Carbs | 58% | 48% |

calculate the quantity of *Cassia* infused.

Leaves from the "senos de mujer" or *Cassia acutifolia* plant, which are actually a sheath-shaped fruit, were acquired from a retail health food store, were packaged without a recognizable brand, and were used by patients for treatment. The sheaths were identified by biologists at the University of Guadalajara. Each had between five and six seeds and displayed characteristics of sun drying.

The amount of water necessary to provide an infusion to each of the rats was added to a glass container. The water was boiled and removed from the oven. The water was then divided according to groups, and either the whole fruit (sheath and seeds) or only the sheaths were added according to the required weight. The latter formulation was due to some patients having reported that only "leaves" should be added. The solution was allowed to cool before being offered to each rat *ad libitum*. Note that each feeder water had a network to prevent pieces of the pod or a seed from obstructing the flow of the infusion.

The animals were randomly divided into two experimental and two control groups. The experimental groups consisted of two subgroups of varying doses, with 6 rats in each subgroup :
· Group F1: 50 mg of fruit in 50 mL of water
· Group F2: 100 mg of fruit in 50 mL of water
· Group S1: 50-mg sheath in 50 mL of water
· Group S2: 100-mg sheath in 50 mL of water
· Control Positive Group: obese and hyperglycemic rats
· Control Negative Group: healthy rats (only to establish the disease model).

Treatment was administered according to the usual practice used for patients. After completion of the 8-week intervention, the rats were fasted for 12 - 16 h and sacrificed under anesthesia.

Blood samples (approximately 5 - 8 mL) were obtained by using cardiac puncture. Blood samples were centrifuged at 1,500 rpm for 10 min, and the supernatant (serum) was collected and stored at -20°C until analysis. Serum glucose concentrations, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine levels to assess biosafety, were determined using enzyme-linked colorimetric techniques. Calculations involving the results were performed with an automated software ERBA 1100 spectrophotometer.

Results were analyzed using SPSS version 21. Data are expressed as means ± standard deviations (SDs). Mann-Whitney U tests were used to assess the disease model, and Kruskal-Wallis tests were used to compare the control

and intervention groups; $P \leq 0.05$ was considered statistically significant.

## 3. Results

We first compared healthy rats (negative control) with untreated obese rats (positive control). Statistical differences between the groups were found in obesity, as defined by elevated body weight, and DM2, as defined by the presence of hyperglycemia and the polydipsia, polyphagia, and polyuria triad (Fig. 1). We observed an additional 50% increase in body weight between the beginning and the end of the study ($P = 0.004$). Similarly, we found differences in glucose levels above 100 mg/dL ($P = 0.03$). For the clinical triad of diabetes, the difference was both statistical and clinical ($P = 0.004$).

The biosafety parameters were analyzed following hypercaloric diet consumption. To assess the safety of the disease model and monitor other conditions that might compromise the study, we measured the creatinine levels to monitor renal biosafety and the ALT and the AST levels to evaluate liver biosafety. We did not detect any clinical or statistical differences ($P > 0.05$) in these parameters (Fig. 2), suggesting that the model could be accepted and would have no adverse side effects on the liver or the kidneys. These observations allowed us to assess the biosafety of the intervention in this study.

The effects of *Cassia acutifolia* intervention on body weight and glucose levels in obese diabetic rats were measured, and the results are presented in Fig. 3. That figure shows the homogeneity of the rats in terms of body weight at the start of treatment, thus confirming that the body weights of the rats could be meaningfully compared. The figure also shows statistical differences, $P \leq 0.05$, in the final weights compared to the baseline weights and in the final glucose levels compared to the baseline levels. Fig. 4 displays the biosafety parameters of the various treatments compared with those of the control group. The three biosafety parameters were elevated in the treatment groups compared with those in the control groups ($P <$
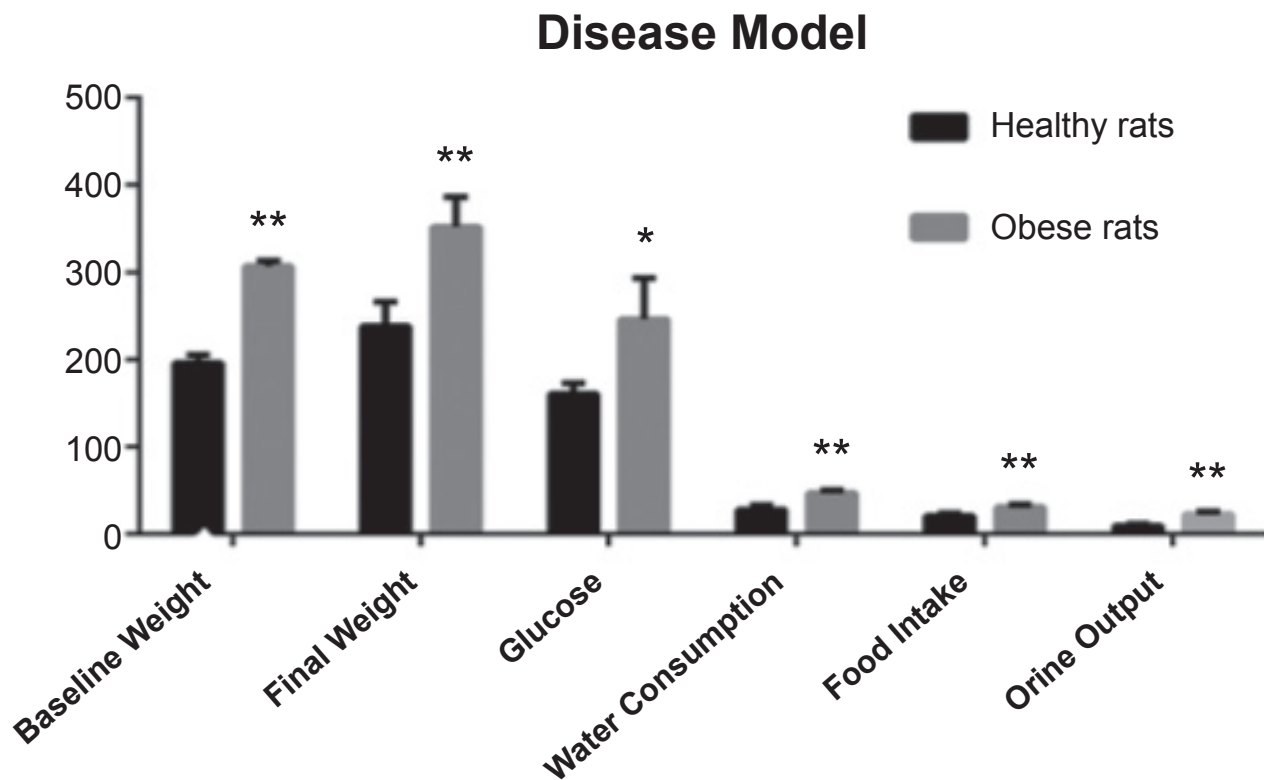


**Figure 1** Differences are seen between the clinical data that allow us to define the presence of obesity and DM2. The values are expressed as means + SDs. Baseline and final weight are in g, serum glucose level in g/dL, water consumption in mL, food intake in g, and orine output counting in mL, all of which are means per day. * = $P \leq 0.05$, and ** = $P \leq 0.01$.

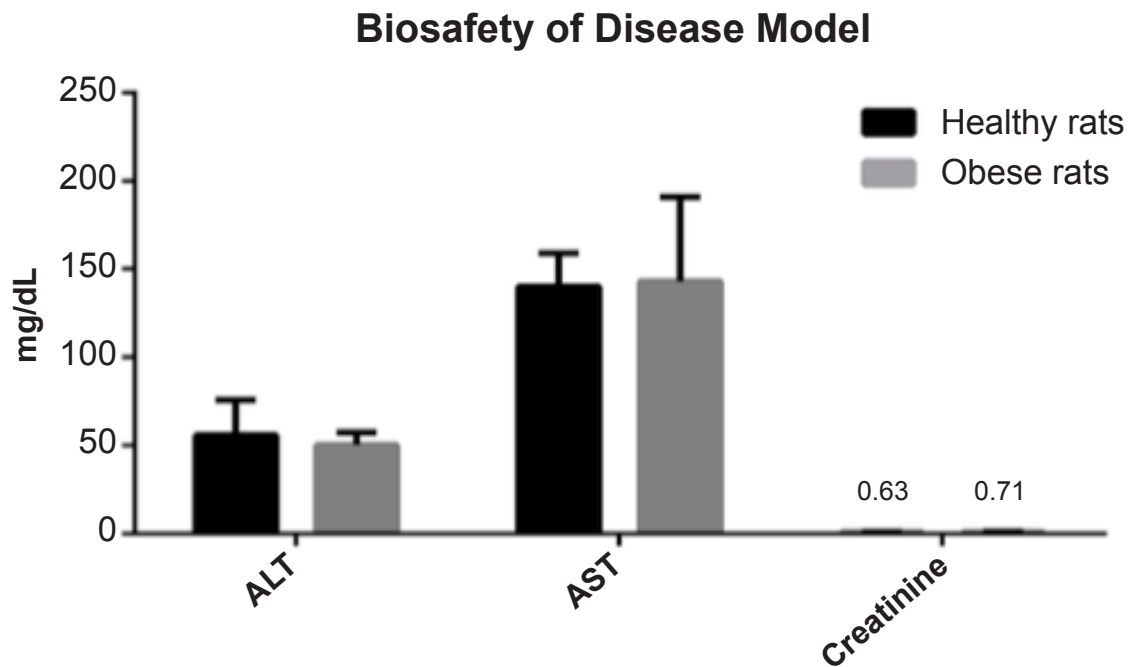DM2, Type 2 diabetes mellitus; SDs, standard deviations.

## Biosafety of Disease Model



**Figure 2** No statistical differences were observed, the disease model used in this study was biosafety. The values are expressed as means + SDs.

SDs, standard deviations; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

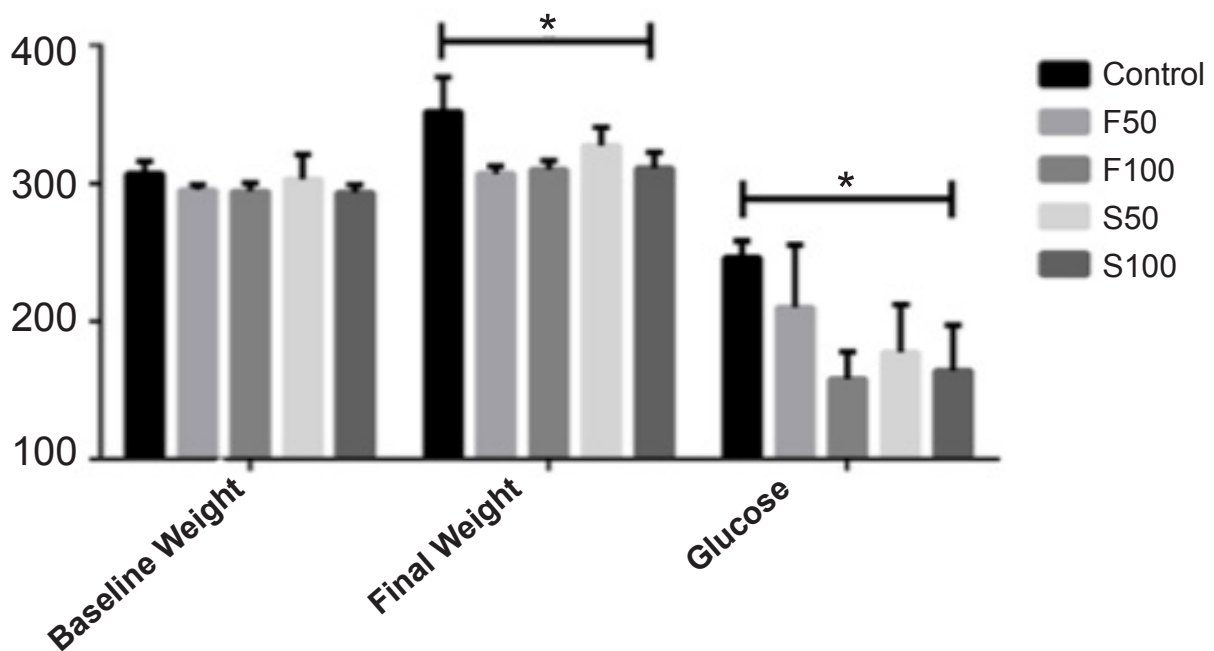## Comparison of clinical values between intervention groups and control group



**Figure 3** The weight and glucose level are seen to decrease. The values are expressed as means + SDs. * = $P < 0.05$, and ** = $P < 0.01$.
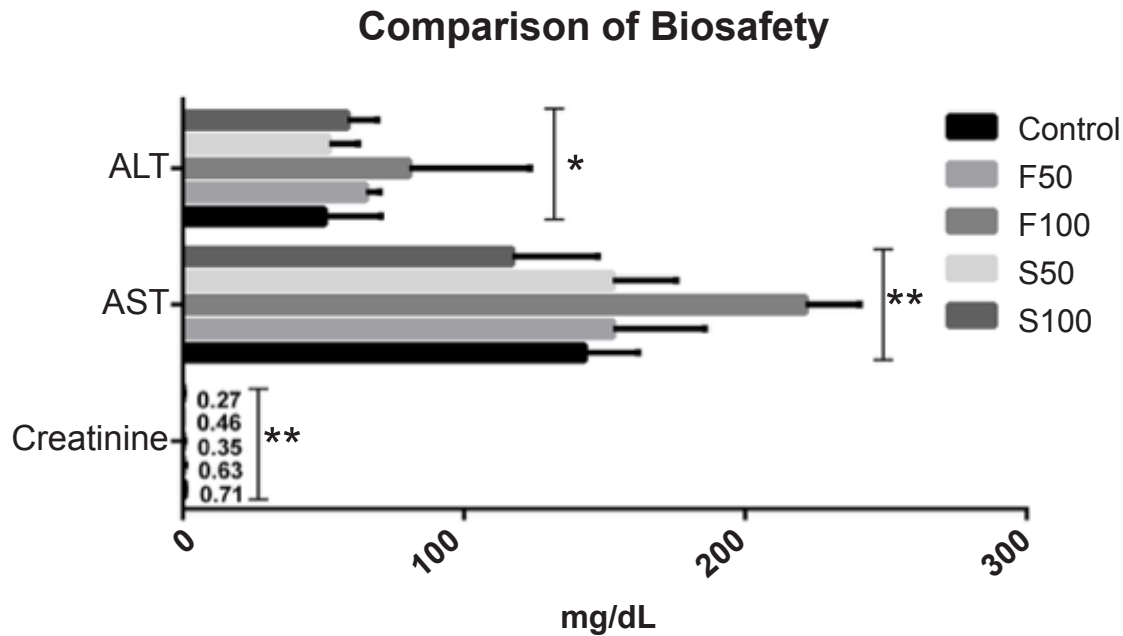
SDs, standard deviations.

## Comparison of Biosafety



**Figure 4** The intervention shows an unsafe effect. The values are expressed as means + SDs. *P = < 0.05, and **P = < 0.01.

SDs, standard deviations; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

0.05), indicating that *Cassia acutifolia* may be unsafe for consumption.

## 4. Discussion

A high-calorie diet-fed model was used to feed rats in order to generate obesity and DM2 that were consistent with clinical parameters. We generated a rat model that closely resembled what occurs in patients under these conditions. Specifically, a 50% increase in body weight was observed. Additionally, glucose levels were elevated by more than 100 mg/dL, in contrast to the negative control, and we observed the presence of polydipsia, polyuria and polyphagia ($P < 0.05$). One of the problems we observed was the variability of the glucose results. Hyperglycemia was present in all hypercaloric rats, with values ranging from 200 mg/ dL to approximately 340 mg/dL, which could compromise statistical attempts to compare our results with other studies. Despite this, the disease model was used to evaluate the impact of *Cassia acutifolia* on obesity and DM2 in a rat model.

Compared with other models, the model used in this study was inexpensive and did not require chemical additives, making it a safer model. Specifically, although models enriched with lipids or toxic agents that damage alternate organs, such as the streptozotocin or the alloxan model, may closely simulate DM2, they may also generate side effects. Furthermore, those models do not include

the impact of obesity [5, 6]. In contrast, the model used in this study was sufficient to evaluate the effect of *Cassia acutifolia*, which generated anti-obesogenic and glucose regulatory effects. However, treatment also generated liver damage, including transaminase levels greater than twice the normal levels. Additionally, ALT levels in the intervention groups, especially in the 100 mg/50 mL fruit group, were more than double the normal range observed in the control rats. This suggests that *Cassia acutifolia* has an adverse component, particularly for the seeds. Moreover, a decrease in creatinine levels in the intervention groups was observed. This could be explained by increased body weight and decreased physical activity, which could reduce muscle volume and activity. However, future studies should address this point, as *Cassia* could have a myotoxic component, and this study did not assess muscle volume or activity.

The anti-obesogenic effect observed was based on mitigation of the weight increase observed in the positive control group. However, weights were not fully restored to the baseline values. Thus, although the effect was statistically significant, it was not clinically significant. This could be explained by the presence of sennosides, the most widely studied compound in the *Cassia* plant. Posadzki in 2013 [7] conducted a systematic review investigating the adverse effects of medicinal plants. That review reported a laxative effect of sennosides. However, that finding is controversial, as the same plant has been reported possibly to cause constipation. The systematic review referenced a study by Ul-

bricht *et al.* in 2011 [8], which is another systematic review focused only on *Cassia*. Her review reported the various symptoms for this plant and the resulting effects; it also indicated sennosides as the main components. The author noted a laxative, but not an anti-obesogenic effect. However, no effect on the glucose level was reported.

In this study, a decrease in glucose levels was observed in rats administered *Cassia*. Administration of 50 mg/50 mL of the fruit had the lowest effect, with a mean decrease of 30 mg/dL. However, treatment with 100 mg/50 mL of either the fruit or the sheath resulted in an average decrease of approximately 90 mg/dL ($P$ = 0.01). Thus, the higher dose had a greater effect on glucose levels. This indicates that treatment with this infusion that many patients ingest has an anti-hyperglycemic effect. As studies investigating *Cassia* are limited and no studies, to the best of our knowledge, have investigated the impact of *Cassia* on glucose levels, the reasons for this result remain unclear. The laxative effects would not explain the results obtained, and the antioxidant effect reported in the aforementioned systematic reviews cannot explain the effects on glucose.

These findings warrant further investigation of this plant for medicinal use. Although it may cause adverse effects, beneficial effects may be achieved if the necessary compounds can be isolated and extracted. However, previous research investigating the use of this plant, or its active components except for the sennosides, for the treatment of patients with diabetes has not been sufficient.

## 5. Conclusion

*Cassia acutifolia* has anti-hyperglycemic effects in obese diabetic rats; however also has adverse effects; further studies are needed on its components and routes of action. Currently, it should not be administered to patients.

## Acknowledgment

## Conflict of interest

The authors declare that they have no conflicts of interest.

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