Hypoglycemic and Antihyperlipidemic effects of *Syzygium cumini* (Lamarck) Skeels and *Syzygium paniculatum* (Gaertn.)

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**Summary**

**Introduction:** there is a recent increase in interest in the use of medicinal and phytotherapeutic plants. **Objective:** to verify the effect of ethanolic extracts from different parts of fruits of two species of the genus Syzygium, on glucose and lipid levels in the blood, using animal models. **Results:** the bark extract of the seeds of *S. cumini* (Lamarck) Skeels proved to be effective in reducing blood glucose levels. This same extract was also effective in lowering blood cholesterol levels. The extract from the seed nuclei of *S. cumini* (Lamarck) Skeels and *S. paniculatum* (Gaertn) were effective in reducing blood triglyceride levels. Extracts from all parts of the fruits of *S. cumini* (Lamarck) Skeels and of the seed nuclei of *S. paniculatum* (Gaertn) prevented weight gain in the animals. **Conclusion:** in general, the seed extract of both species showed a direct influence on the parameters and characteristics under study.

**Keywords:** Diabetes, *Syzygium*, Lipids, Blood glucose.
Resumen

Efectos hipoglucémicos y antihiperlipidémicos de *Syzygium cumini* (Lamarck) Skeels y *Syzygium paniculatum* (Gaertn.)

**Introducción:** hay un reciente aumento del interés en el uso de plantas medicinales y fitoterapéuticas. **Objetivo:** verificar el efecto de los extractos etánolícos de diferentes partes de frutos de dos especies del género *Syzygium*, sobre los niveles de glucosa y lípidos en la sangre, utilizando modelos animales. **Resultados:** el extracto de corteza de las semillas de *S. cumini* (Lamarck) Skeels demostró ser eficaz para reducir los niveles de glucosa en la sangre. Este mismo extracto también fue eficaz para reducir los niveles de colesterol en la sangre. El extracto de los núcleos de semillas de *S. cumini* (Lamarck) Skeels y *S. paniculatum* (Gaertn.) fueron eficaces para reducir los niveles de triglicéridos en la sangre. Los extractos de todas las partes de los frutos de *S. cumini* (Lamarck) Skeels y de los núcleos de semillas de *S. paniculatum* (Gaertn.) impidieron el aumento de peso en los animales. **Conclusión:** en general, el extracto de semillas de ambas especies mostró una influencia directa sobre los parámetros y características en estudio.

*Palabras clave:* Diabetes, *Syzygium*, lípidos, glucemia.

Resumo

Efeitos hipoglicemiantes e antihiperlipidêmicos de *Syzygium cumini* (Lamarck) Skeels and *Syzygium paniculatum* Gaertn

**Introdução:** há um aumento recente do interesse pelo uso de plantas medicinais e fitoterápicas. **Objetivo:** verificar o efeito de extratos etanólicos de diferentes partes de frutos de duas espécies do gênero *Syzygium*, sobre os níveis de glicose e lipídios no sangue, por meio de modelos animais. **Resultados:** o extrato da casca das sementes de *S. cumini* (Lamarck) Skeels mostrou-se eficaz na redução da glicemia. Este mesmo extrato também foi eficaz na redução dos níveis de colesterol no sangue. Os extratos dos núcleos das sementes de *S. cumini* (Lamarck) Skeels e *S. paniculatum* (Gaertn) foram eficazes na redução dos níveis de triglicerídeos no sangue. Extratos de todas as partes dos frutos de *S. cumini* (Lamarck) Skeels e dos núcleos das sementes de *S. paniculatum* (Gaertn) impediram o ganho de peso nos animais. **Conclusão:** em geral, o extrato de sementes de ambas as espécies apresentou influência direta nos parâmetros e características em estudo.

INTRODUCTION

Type 2 diabetes mellitus is a heterogeneous syndrome, characterized by a progressive decline of insulin action followed by the inability of β-pancreatic cells to compensate this resistance to insulin. According to the Brazilian Diabetic Society, the number of adults with diabetes mellitus in the world in 2017 was estimated at around 425 million and is expected to rise to 629 million by 2045 [1].

About 79% of these individuals with diabetes mellitus live in developing countries, where the diabetes mellitus epidemic is more intense and shows a growing proportion of affected people in younger age groups [2].

According to Barreiro et al. [3], learning within different ethnic groups resulted in priceless contributions to the development of research in natural products and knowledge of the close relationship between the chemical structure of a certain substance and its biological properties. The World Health Organization has provided incentives for research about the technical-scientific knowledge of medicinal plants due to the increasing number of people currently employing their properties [4].

With the increased interest in using medicinal phytotherapeutic plants, the purpose of this paper was to verify the effect of ethanolic extracts of different parts from the fruits of two species of the Syzygium genus on blood glucose and lipid levels and other metabolic alterations typical of diabetes mellitus, using animal models.

MATERIAL AND METHODS

Animals

Male Wistar rats, 35 days old, weighing 140 ± 28.07 g, were kept in individual metabolic cages, at controlled temperature (22 ± 2 °C), with dark/light cycles of 12/12 h. All animals were given a standard commercial diet and water ad libitum prior to the diabetes induction. The specimens were acquired from the Multidisciplinary Center of Biological Investigation of the State University of Campinas, Brazil (Cemib/Unicamp). The experiment was carried out in the facilities of the Department of Veterinary Medicine of the Federal University of Lavras, Brazil. The experimental protocol nº 23101.003106/2013-55 was submitted and approved by the Committee for Ethics in Animal Research of the Federal University of Tocantins, Brazil.
Administration of the high-lipid diet and diabetes induction

The animals were submitted to the *ad libitum* intake of a semi-purified diet with high lipid contents, based on the guidelines of the American Institute of Nutrition (AIN-93G) [5-7], with modifications. The overall energy component of the diet consisted of 44% lipids, 15.2% proteins and 40.8% carbohydrates.

Composition of the High-Lipid Diet (g100g⁻¹): corn starch 24.0; dextrinized corn starch (90-94% of tetrassacarides) 13.0; casein (≥ 85% of protein) 20.0; soybean oil 7.0; pork fat 15.0; cellulose 5.0; mineral pre-mix AIN-93G 3.5; vitamin pre-mix AIN-93 1.0; L-cystine 0.3; DL-methionine 0.16; choline bitartrate 0.25; cholesterol 0.5; sodium cholate 0.2; butylhydroxytoluene 0.01(BHT); vitamin E 0.01.

The animals were given an intraperitoneal injection of streptozotocin (50 mg/Kg) (Sigma-Aldrich), having been offered no food during the previous night (12 h). After this procedure, the animals were initiated in the high-lipid diet. Three days after the streptozotocin injection, the glucose blood levels were measured daily for seven days, using Accu-Check Advantage II (Roche Diagnostics, Germany). Animals with fasting glucose higher or equal to 200 mg/dL were considered diabetic and randomly included in the study groups. The animals were given the high-lipid diet and water *ad libitum* until the end of the experiment.

Collecting the plant material

The plant material was collected in the morning (ambient temperature: 21.6 °C), at the Historical Campus of the Federal University of Lavras (UFLA), in Lavras, Minas Gerais, Brazil and at the São Bento Farm, in Três Corações, Minas Gerais, Brazil. The species *Syzygium cumini* (L.) Skeels and *Syzygium paniculatum* (Gaertn.) were properly identified by Dsc. Marcos Sobral, at the Federal University of São João Del Rei, in São João Del Rei, Minas Gerais, Brazil, and registered in the Herbarium of the UFLA Biology Department at numbers 25,133 and 25,134, respectively. The plant material was processed on the same day. The different parts of the fruits were stored in a ventilated drier with mechanical air circulation (Fanem 320-SE), at 30 °C, for approximately 7 days. After this drying period, the ethanolic extracts were prepared using cold extraction.

Obtaining the extracts

The extraction procedure was carried out at the Laboratory of Organic Chemistry of the Federal University of Lavras, where the different parts of the plants were sorted and dried and then submitted to cold extraction with 98% ethanol [8]. Extracts were prepared with the seeds, the pulp and the seed skins of each of the two species. The material was submitted to particulation, submerged in 98% ethanol, stored in Erlenmeyer,
wrapped in aluminum foil and stored at room temperature for 4 days. After this period, the plant material was vacuum filtered using a Buchner funnel. The solvent was evaporated using a rotating evaporator (Büchi R-114) and hot water bath (Büchi B-480), at 50 °C. The extract thus obtained was placed on previously dried and weighed porcelain capsules and left in a non-ventilated drier at 45 °C until a constant weight was obtained. After the total evaporation of the solvent, the capsules were wrapped in a layer of plastic film and another of aluminum foil and left in the fridge, at temperatures between 3 and 4 °C.

**Biological assay**

Diabetes was induced in the animals according to the methodology proposed by Reeves *et al.* [7] and Srinivasan *et al.* [9], who advocated the use of the high-lipid diet associated to streptozotocin (50 mg/kg). Each group consisted of 6 animals (n= 42), initially treated with high-lipid diet and streptozotocin, and later distributed randomly among the groups. Group A: diabetic animals, not treated (control); Group B: diabetic animals treated with the alcoholic extract of seed cores of *S. paniculatum* (Gaertn.) (SP seeds); Group C: diabetic animals treated with the alcoholic extract of the pulp of *S. paniculatum* (Gaertn.) (SP pulp); Group D: diabetic animals treated with the alcoholic extract of the seed skins of *S. paniculatum* (Gaertn.) (SP skin); Group E: diabetic animals treated with the alcoholic extract of the seed skins of *Syzygium cumini* (L.) Skeels (SC skin); Group F: diabetic animals treated with the alcoholic extract of the pulp of *Syzygium cumini* (L.) Skeels (SC pulp); Group G: diabetic animals treated with the alcoholic extract of seed cores of *Syzygium cumini* (L.) Skeels (SC seeds).

The doses of the extracts were dissolved in 0.5 mL of drinking water, Ingá brand, coming from the same batch, and the resulting solution was given by gavage once a day for 20 days. A flexible tube with a rounded tip was introduced into the animal’s mouth and gently pushed through the esophagus to the stomach. The choice of doses used for each extract was based on the available literature concerning studies of the hypoglycaemic effect of *Syzygium* plant genus and was 200 mg/kg/day for all extracts tested. Food and water intake and urine volume were measured every day. The experiment lasted 21 days. Blood was collected on days 0, 7 and 14, by means of the amputation of the tip of the tail, for the weekly biochemical tests. At the end of the experiment (day 21), euthanasia was done, the animals were put down after anesthesia with sodium thiopenthal (50 mg/kg) and were put to exsanguination [10]. Blood was collected through cardiac puncture for biochemical testing. The livers and pancreases were sent out for anatomicopathological examination.
Evaluation of the biochemical parameters

The capillary level of fasting glucose was evaluated on a daily basis until the diabetes mellitus was clinically identified. For this proceeding, the fasting glucose was assessed on a weekly basis, using a colorimetric enzymatic test performed with commercial kits (Labtest Diagnóstica-SA). Insulin levels were measured before the beginning and at the end of the treatment period, using an Elisa commercial kit manufactured by Milipore®. Triglyceride levels were measured weekly using a colorimetric test performed with commercial kits (Labtest Diagnóstica-SA). The weekly evaluation of cholesterol levels was carried out with colorimetric enzymatic test kits (Labtest Diagnóstica - SA). Total cholesterol, fractions HDL and LDL+ VLDL were measured.

Histological testing

After the cardiac puncture, the animals were put down and had their abdominal cavity opened to expose the internal organs. Fragments of pancreas and liver were collected, stored in formaldehyde 10 % and processed by inclusion in paraffin. Sections 5.0 μm thick were colored with hematoxylin-eosin and studied by optical microscopy.

Experimental outline

The design of the experiment was the completely randomized type (CRS), with six repetitions. The treatments were arranged in different groups (A, B, C, D, E, F and G); body weight, water intake, food intake and urinary volume were measured daily, and the biochemical parameters were measured weekly (days 0, 7, 14 and 21).

Statistical Analysis

The data were submitted to the Shapiro-Wilk normality test. Those that did not present a normal distribution (p < 0.05) were transformed in order to obtain normality (square root) and analyzed by parametric statistic tests (Variance Analysis, Student’s t-test and paired t-test). Those figures that even after transformation did not reach normal distribution, had their transformations disregarded and were analyzed by means of non-parametric statistic tests (Kruskal-Wallis and Dunn’s test). The statistical analyses were performed using the Statistical Analysis System-SAS, version 9.2 [11].

Results and discussion

Insulin amounts did not differ significantly within the same treatment when compared before and after the experimental period; a significant difference was found only for the control group. As it can be seen, the control group showed a significant increase in insulin amounts before and after the treatment.
Table 1. Average figures for blood insulin and glucose before and after the experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood insulin (ng/dL) period</th>
<th>Blood glucose (mg/dL) period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>2.39 ± 0.33*</td>
<td>3.59 ± 0.55*</td>
</tr>
<tr>
<td>SP Seeds</td>
<td>3.85 ± 0.47</td>
<td>3.60 ± 0.32</td>
</tr>
<tr>
<td>SP Pulp</td>
<td>3.55 ± 0.59</td>
<td>3.36 ± 0.50</td>
</tr>
<tr>
<td>SP Skin</td>
<td>3.12 ± 0.21</td>
<td>3.36 ± 0.65</td>
</tr>
<tr>
<td>SC Skin</td>
<td>3.68 ± 0.21</td>
<td>3.71 ± 0.61</td>
</tr>
<tr>
<td>SC Pulp</td>
<td>2.30 ± 0.03</td>
<td>2.30 ± 0.03</td>
</tr>
<tr>
<td>SC Seeds</td>
<td>2.28 ± 0.03</td>
<td>2.30 ± 0.07</td>
</tr>
</tbody>
</table>

*Differed significantly before and after the experimental period (p < 0.05) according to the paired t-test.

The results obtained for blood insulin in this study go against those of the authors who state that *S. cumini* (L.) Skeels has hypoglycemic effects due to an increase in the plasmatic levels of insulin [12-14]. However, they are similar to those of Vikrant *et al.* [15], who observed a preventive effect for the development of hyperinsulinemia in rats fed with fructose, during their study of the ethanolic extracts of *S. cumini* (L.) Skeels.

No significant differences were observed in terms of fasting glycaemia when comparing the control and the treatment groups. Only on the 7th day after treatment the glycaemia of SC Pulp and SC Seeds treatment groups appeared significantly higher (p < 0.05) than SP pulp group.

Regarding the amounts for fasting glucose, in the different treatment days within a same group, a statistically significant difference was observed only in SP Pulp group, between the glycaemia after 7 days of treatment and the final glycaemia (21 days). A significant increase in glucose levels was observed at the end of the experimental period. Similarly, other authors also failed to observe an effect of *S. cumini* (L.) Skeels on the reduction of glucose levels in laboratory animals or humans [16-18].

Table 2. Average percentage of fasting glucose, cholesterol and triglycerides reduction/increase in each treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycaemia (mg/dL) percentage’s increase or reduction</th>
<th>Cholesterol (mg/dL) percentage’s increase</th>
<th>Triglyceride (mg/dL) Percentage’s reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.56 %</td>
<td>229.46 %</td>
<td>-54.39 %</td>
</tr>
<tr>
<td>SP Seeds</td>
<td>46.41 %</td>
<td>122.93 %</td>
<td>-60.81 %</td>
</tr>
</tbody>
</table>

(keep going)
Biological effects of *Syzygium cumini* Skeels and *Syzygium paniculatum*

<table>
<thead>
<tr>
<th></th>
<th>SP Pulp</th>
<th></th>
<th>SC Skin</th>
<th></th>
<th>SC Pulp</th>
<th></th>
<th>SC Seeds</th>
<th></th>
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</tr>
<tr>
<td></td>
<td>31.94 %</td>
<td></td>
<td>284.18 %</td>
<td></td>
<td>-28.95 %</td>
<td></td>
<td>-15.64 %</td>
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</tr>
<tr>
<td></td>
<td>89.51 %</td>
<td></td>
<td>24.28 %</td>
<td></td>
<td>-46.43 %</td>
<td></td>
<td>-14.15 %</td>
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<tr>
<td></td>
<td>-38.73 %</td>
<td></td>
<td>14.15 %</td>
<td></td>
<td>-58.12 %</td>
<td></td>
<td>-12.51 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.39 %</td>
<td></td>
<td>62.15 %</td>
<td></td>
<td>-73.28 %</td>
<td></td>
<td>17.41 %</td>
<td></td>
</tr>
</tbody>
</table>

When comparing the differences between the final and initial fasting glucose levels, we observed that all treatment promoted an increase in glycaemia at the end of the experimental period, and that only the SC Skin group saw its glucose levels reduced; its percentage was significantly different from that of the control group.

Groups SP Seed, SP Pulp, SC Seed and SC Pulp showed an increase in the final glucose amounts, yet still inferior to the amounts observed in the control group. This difference was not statistically relevant.

Other studies observed a reduction in glucose levels after the administration of *S. cumini* (L.) Skeels [19-21]. Sharma *et al.* [22], when studying the ethanolic extract of seeds of *S. cumini* (L.) Skeels in rabbits with alloxan-induced diabetes, observed a significant reduction of the fasting glucose, of the hyperglycemic peak in the glucose tolerance test and of the glycosylated hemoglobin.

Our study tested two different parts of the seeds of *S. cumini* (L.) Skeels (skin and core). According to our observations, only the skins of the seeds had any reducing effect on the animals’ fasting glucose, an indication that the active principle is probably located in this part of the seed.

The action mechanism of the jambul fruit in the reduction of glucose levels is not yet clear. However, some works attributed the effects of the plant over blood glucose levels to its presumed inhibition of α-glycosidase and α-amilase, key enzymes in the metabolism of carbohydrates responsible for the final degradation of oligosaccharides and disaccharides, ingested as food, into monosaccharides [20, 23-25].

In the SP Seeds group, blood cholesterol levels increased significantly between the period before the treatment and the 14th day of treatment.

The SC Skin group showed a significant increase of blood cholesterol levels between the period before the treatment and the 14th day of treatment, with a further significant reduction observed between the 14th and 21st days of treatment. The blood levels of triglycerides were significantly reduced between the pre-experiment period and the 21st day of treatment for the SP Seeds and SC Seeds groups.
Table 3. Average figures for blood lipids before and after the experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides (mg/dL)</th>
<th>Total Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control</td>
<td>372.56 ± 97.84</td>
<td>301.12 ± 58.75</td>
</tr>
<tr>
<td>SP Seeds</td>
<td>213.58 ± 110.05*</td>
<td>71.62 ± 21.18*</td>
</tr>
<tr>
<td>SP Pulp</td>
<td>175.43 ± 123.15</td>
<td>101.25 ± 53.19</td>
</tr>
<tr>
<td>SP Skin</td>
<td>229.04 ± 35.61</td>
<td>84.16 ± 52.08</td>
</tr>
<tr>
<td>SC Skin</td>
<td>110.28 ± 88.49</td>
<td>95.68 ± 38.97</td>
</tr>
<tr>
<td>SC Pulp</td>
<td>394.15 ± 84.87</td>
<td>215.04 ± 53.03</td>
</tr>
<tr>
<td>SC Seeds</td>
<td>565.76 ± 61.22•</td>
<td>105.46 ± 54.73•</td>
</tr>
</tbody>
</table>

Values followed by the symbols (*) • differed significantly from each other, by the Dunn Test.

In all treatment groups, including the control, blood cholesterol levels increased, and triglycerides dropped when we compare the serum data between the beginning of the treatment and at the end of it.

Considering the diet with high lipid contents offered ad libitum, it was expected an increase in the blood amounts of both lipids. The percentages of increase for blood cholesterol did not differ significantly from the control group; neither did it differ between the treatments. As for blood triglycerides serum, it must be pointed out that the percentage of reduction observed for the SC Seeds treatment was the highest, yet it was significantly different only from the SC Skin treatment. When comparing the treatments, we observed that the extract of the parts of the seeds of both species were those that showed a significant influence on the lipemia of the animals. Schoenfelder et al. [21], in an experimental study with rats observed hypolipidemic activity in animals with diabetes and improving of the lipid profile.

The action mechanism of these plants in the body remains unknown, but according to literature the effects of S. cumini (L.) Skeels on lipemia can be attributed to different factors, such as the presence of hypocholesterolemic compounds which may work as enzymatic inhibitors, inhibiting cholesterol biosynthesis enzymes, or inhibiting the intestinal absorption of cholesterol, or otherwise inhibiting lipid peroxidation. It is also possible that the extract can stimulate insulin production to the point that the activity of the hormone-sensitive lipase is reduced [13, 21].

No significant differences were observed between the treatments regarding the following biochemical parameters: final total cholesterol, final HDL and final LDL+VLDL (Kruskal-Wallis, at 5 % of significance). As can be observed, the levels of cholesterol of the animals from SP Seeds and SC Skin groups did not exceed the
maximal limits of normality, remaining below 200 mg/dL, even while in the high-
lipid diet. This behavior was not observed in the control groups or in the other groups.

**Table 4.** Average weight gain and food intake for the experimental group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall Weight Gain (grams)</th>
<th>Food Intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.66 ± 17.55</td>
<td>15.36 ± 3.37</td>
</tr>
<tr>
<td>SP Seeds</td>
<td>27 ± 13.51**</td>
<td>15.75 ± 3.66</td>
</tr>
<tr>
<td>SP Pulp</td>
<td>61 ± 27.70</td>
<td>16.91 ± 1.97</td>
</tr>
<tr>
<td>SP Skin</td>
<td>51.66 ± 7.64</td>
<td>16.92 ± 2.30</td>
</tr>
<tr>
<td>SC Skin</td>
<td>26 ± 12.45**</td>
<td>15.22 ± 2.34</td>
</tr>
<tr>
<td>SC Pulp</td>
<td>30 ± 13.42**</td>
<td>16.00 ± 1.96</td>
</tr>
<tr>
<td>SC Seeds</td>
<td>36.66 ± 36.15**</td>
<td>15.74 ± 2.88</td>
</tr>
</tbody>
</table>

** Differed significantly from the control group according to Student’s t-test (p < 0.05).

As can be observed, the control group showed more weight gain than the other
treatment groups. SP Seeds, SC Skin, SC Pulp and SC Seeds groups showed a weight
gain significantly lower than the control group. Food and water intake, urine volume
and hyperglycaemia levels did not differ significantly among the groups according to
the variance analysis. Such evidence is an indication that treatments with the ethanolic
extracts of all parts of the fruits of *S. cumini* (L.) Skeels and of the seeds of *S. paniculatum*
(Gaertn.) may have had a direct influence on the weight of the animals.

Sharma *et al.* [22] observed regulatory effects of the ethanolic extract of the seeds
of *S. cumini* (L.) Skeels on molecules PPARα and PPARγ, which control the energy
and lipid metabolism and are involved in the control of resistance to insulin, adipocyte
differentiation and lipid storage, which may contribute to explaining the smaller
weight gain of the animals in this study.

The histological exams showed no alterations suggestive to toxicity in the liver or pan-
creas tissues analyzed. The livers of all analyzed groups, including the control group,
showed areas of hepatic steatosis, an effect that was attributed to the high-lipid diet.
No areas of necrosis were observed. None of the animals in the treated groups died.

Sridhar *et al.* [14], when administering ethanol seeds of *S. cumini* (L.) Skeels to dia-
etic animals, in the form of powder, did not report any abnormality or mortality, there-
fore suggesting that this plant has low toxicity. Sharma *et al.* [22], when administering
the ethanolic extract of seeds of *S. cumini* (L.) Skeels to laboratory animals, in the same
dose used in this study, did not observe any modification of the biochemical param-
eters suggestive to toxicity.
Overall, the seed extracts from both species were those that showed a direct influence on the parameters and characteristics under study.

It is known that plant chemical substances may vary according to factors such as humidity, sun exposure and climate conditions. The failure to observe some metabolic effects in this study does not rule out the possibility that these effects may be obtained with plants collected in another period of the year or in different geographical locations.

Disclosure statement

No potential conflict of interest was reported by the authors.

References


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