Hematological and pathological effects of 0.25% purified simmondsin in growing rats

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Abstract

Simmondsin, a glycoside extracted from jojoba meal (Simmondsia chinensis), causes a reduction in food intake after oral administration. To investigate whether a moderate dose of simmondsin, inducing a food intake reduction of about 25%, has transient or permanent detrimental effects on hematological and pathological parameters in rats, the following study was conducted. Rats treated with simmondsin (0.25% mixed in the food) were compared to the appropriate control- and pair-fed rats. After 10 weeks, 50% of the rats receiving simmondsin or pair-fed to simmondsin treated rats were changed to a control diet ad libitum, the other 50% remained on the simmondsin or pair-fed treatment until week 20. Growth, food intake, hematological and some pathological parameters were determined. From this study it could be concluded that simmondsin treatment induced a transient increase in organ weights compared to pair-fed rats, and a slight macrocytic, normochromic anemia, that also recovered completely after withdrawal of simmondsin. However, the conducted study did not reveal any microscopic or biochemical sign of toxicity. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Simmondsin; Rats; Toxicity

1. Introduction

Since Booth et al. (1974) published the results of their experiments on jojoba, it is a known fact that simmondsin reduces food intake. Cokelaere et al. (1995) demonstrated that simmondsin exerts its food intake reducing effect by inducing satiation and not by its toxicity. No overt signs of toxicity were observed (Cokelaere et al., 1992a; Flo et al., 1998), at the concentrations of simmondsin (0.15–0.25%) used to induce a food intake reduction of about 20%. The question remains, however, whether simmondsin has any
toxic effect that could explain the variable mortality observed in rodents fed high concentrations of jojoba meal in food with a low protein content (Verbiscar et al., 1980). A small but significant anemic reaction was observed in rats after 11 weeks of treatment with 3% defatted jojoba meal (Cokelaere et al., 1993), but it has not been determined whether or not these effects are due to simmondsin or to other compounds present in the meal.

Therefore, a study was conducted with the following aims: (1) to look for a possible anemic reaction after the administration of 0.25% purified simmondsin; and (2) to study the effect of purified simmondsin on the structure and function of the internal organs in rats.

2. Material and methods

In this study, 160 young male Wistar rats were housed, two by two, in plastic cages under standard laboratory conditions (room temperature of 20 ± 2°C; 12 h light exposure: lights on at 08:00 h). The rats were divided into three groups: (1) a control group, C, containing 40 rats that had free access to normal rat chow (Carfil Q, Belgium) fed in mangers specially designed to avoid food spilling; (2) a simmondsin group, S, containing 60 rats that were offered normal rat chow supplemented with 0.25% of purified simmondsin; and (3) a pair-fed group, P, containing 60 rats that were pair-fed to the simmondsin group, which means that they received daily, the same amount of food as eaten by the simmondsin group on the previous day. All the animals had free access to tap water. Food intake was measured every 2 or 3 days, by weighing the mangers, and the animals were weighed weekly.

Four rats per group were sacrificed at 09:00 h at the end of weeks 1, 2, 3, 5, 7 and 10 for hematological and pathological examination.

After the 10th week, the remaining 36 animals in the S and P groups were divided in two groups of 18 rats each. The first group of S and P rats, were continued on the original treatment, the second groups of rats were re-fed, i.e. had free access to control food (RS and RP groups, respectively). The 16 remaining control rats (C) continued on control food ad libitum. The food intake and body weight were followed as described above. At weeks 12, 15, 18 and 20, four rats per group were anesthetized with ether for examinations.

At necropsy, blood was collected by cardiac puncture, and blood and bone marrow smears were made for microscopic examination. Hematological parameters were measured using an automated blood cell counter machine (Abbott Cell-Dyn® 1300; Abbott Diagnostic Divisions, USA). More specifically, the number of red blood cells per μl (RBC), the hemoglobin concentration (Hgb), the hematocrit value (HTC), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), the number of white blood cells (WBC) and platelets (PT) per μl were measured. Internal organs were dissected and the weight of heart, lungs, liver, pancreas, left kidney, thymus and spleen were recorded. The organs were fixed in 10% neutral buffered formalin solution for pathological evaluation. In addition to the above organs the stomach, small and large intestine, the cecum and the mesenteric lymph nodes in the vicinity of the cecum were also collected for evaluation. Serum samples were analyzed for liver and kidney function (GOT, GPT, creatinin) and for possible hemolysis (lactate dehydrogenase (LDH), bilirubin).

The simmondsin used was obtained from Dr Tom Abbott of the USDA laboratories in Peoria (USA) and was recrystallized once in a methanol/acetone mixture to obtain a 99%+ pure fraction.

Results are expressed as mean ± S.E.M. and statistical analysis was performed using ANOVA with a Tukey–Kramer post-test for significance. Values were considered significant when P < 0.05.

3. Results and discussion

Fig. 1 shows that the food intake in the simmondsin treated rats for the entire period was about 75% of that in the control rats, consistent with previous experiments (Cokelaere et al., 1992a). During the second 10 weeks period, the
RS and RP rats increased their food intake immediately to about 90% of that in the control rats and reached about 95% of that intake at the end of the experiment. In addition, the RP rats showed a huge food intake increase during the first week of re-feeding. It has to be taken into account that these rats were conditioned to eat their daily ration in a few minutes, as they received a reduced amount of food daily. This increased their food intake rate. Therefore this extremely high food intake the first week might have been due to the fact that they were allowed to eat throughout the entire day.

The increase in body weight (Fig. 2) observed in rats of the simmondsin- and pair-fed groups was lower than that of the control rats, due to the lower food intake. This is clear from the fact that there was no difference in the body weight between the simmondsin treated and pair-fed groups. After re-feeding, both RS and RP animals gained weight faster than the S and P animals continuing the original treatment.

Hematological parameters varied from week 3 on (data not shown). This trend continued for the entire 20-week period (Table 1). RBCs became less numerous in the simmondsin group, S, compared to the control, C, and pair-fed, P, groups. When simmondsin was withdrawn after week 10, the RBC count increased again and was normal by week 20. The same pattern was observed for the hemoglobin concentration and the HTC.

The rats in the pair-fed group, P, showed a somewhat higher hemoglobin concentration and HTC compared to the rats in the control group, which can not be fully explained. It is likely associated with the amount of food eaten or with the food intake pattern, as one observed that these differences disappear after re-feeding. Since the fluid intake of rats is closely linked to their food intake (Fitzsimons and Le Magnen, 1969), pair-fed rats should drink less than free feeding rats, and therefore may have a somewhat lower blood serum volume than the control rats. But this remains merely speculative.

MCV of the RBCs increased with simmondsin treatment, and returned to normal after withdrawal of simmondsin. This increase in RBC volume is also reflected in an increase in the MCH or amount of hemoglobin per cell. In contrast, the MCHC or the concentration of hemoglobin in the blood retained in the red cells, remained constant (Table 2).
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<td>P</td>
<td>RS</td>
<td>RP</td>
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<td>RBC (M/μl)</td>
<td>7.52 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.70 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.15 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RBC (M/μl)</td>
<td>7.75 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.27 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.14 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.57 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.78 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>WBC (K/μl)</td>
<td>3.05 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.30 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>WBC (K/μl)</td>
<td>8.75 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.30 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.68 ± 0.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.32 ± 1.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.47 ± 1.36&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Platelets (K/μl)</td>
<td>971 ± 17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>883 ± 42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Platelets (K/μl)</td>
<td>997 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>872 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1000 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>903 ± 18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>935 ± 37&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>Hgb (g/dl)</td>
<td>14.9 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.8 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.6 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Hgb (g/dl)</td>
<td>15.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.7 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Htc (%)</td>
<td>37.9 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.4 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.2 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Htc (%)</td>
<td>38.9 ± 0.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>35.1 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.6 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.5 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>MCV (fl)</td>
<td>50.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.3 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.8 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MCV (fl)</td>
<td>50.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.0 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>MCH (pg)</td>
<td>19.8 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>MCH (pg)</td>
<td>19.8 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.1 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.9 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>MCHC (g/dl)</td>
<td>39.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MCHC (g/dl)</td>
<td>39.5 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.4 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.7 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.1 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.2 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup>Values indicate mean ± S.E.M. Different superscripts indicate a significant difference between different treatments on the same sampling time (P-value < 0.05).
Table 2
Relative organ weight of growing male Wistar rats treated with simmondsin (0.25%) (S) for 10 and 20 weeks, compared to control rats (C), pair-fed rats (P) and rats that are re-fed after simmondsin treatment (RS) or pair-feeding (RP) *

<table>
<thead>
<tr>
<th>10 weeks</th>
<th>C</th>
<th>S</th>
<th>P</th>
<th>20 weeks</th>
<th>C</th>
<th>S</th>
<th>P</th>
<th>RS</th>
<th>RP</th>
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<tbody>
<tr>
<td>Heart</td>
<td>0.30 ± 0.01b</td>
<td>0.37 ± 0.01a</td>
<td>0.33 ± 0.02ab</td>
<td>Heart</td>
<td>0.26 ± 0.01b</td>
<td>0.31 ± 0.01a</td>
<td>0.27 ± 0.01b</td>
<td>0.28 ± 0.01b</td>
<td>0.24 ± 0.01b</td>
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<tr>
<td>Lungs</td>
<td>0.38 ± 0.02b</td>
<td>0.56 ± 0.04a</td>
<td>0.42 ± 0.03b</td>
<td>Lungs</td>
<td>0.34 ± 0.01b</td>
<td>0.51 ± 0.04a</td>
<td>0.34 ± 0.02b</td>
<td>0.40 ± 0.03b</td>
<td>0.35 ± 0.01b</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.29 ± 0.02b</td>
<td>0.39 ± 0.02a</td>
<td>0.36 ± 0.01ab</td>
<td>Pancreas</td>
<td>0.30 ± 0.03</td>
<td>0.36 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.20 ± 0.02b</td>
<td>0.24 ± 0.01a</td>
<td>0.21 ± 0.01ab</td>
<td>Spleen</td>
<td>0.16 ± 0.01b</td>
<td>0.20 ± 0.01a</td>
<td>0.16 ± 0.01b</td>
<td>0.19 ± 0.01ab</td>
<td>0.17 ± 0.01ab</td>
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<tr>
<td>Left kidney</td>
<td>0.32 ± 0.02b</td>
<td>0.36 ± 0.01a</td>
<td>0.33 ± 0.01b</td>
<td>Left kidney</td>
<td>0.29 ± 0.02b</td>
<td>0.38 ± 0.01a</td>
<td>0.29 ± 0.01b</td>
<td>0.34 ± 0.01a</td>
<td>0.28 ± 0.01b</td>
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<tr>
<td>Liver</td>
<td>2.87 ± 0.10b</td>
<td>3.30 ± 0.11a</td>
<td>2.76 ± 0.05b</td>
<td>Liver</td>
<td>2.61 ± 0.14b</td>
<td>3.33 ± 0.08a</td>
<td>2.56 ± 0.04b</td>
<td>2.83 ± 0.10b</td>
<td>2.53 ± 0.07b</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.142 ± 0.008</td>
<td>0.142 ± 0.006</td>
<td>0.122 ± 0.002</td>
<td>Thymus</td>
<td>0.079 ± 0.005ab</td>
<td>0.072 ± 0.002b</td>
<td>0.070 ± 0.003b</td>
<td>0.076 ± 0.024ab</td>
<td>0.092 ± 0.005ab</td>
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* Values indicate mean ± S.E.M. Different superscripts indicate a significant difference between different treatments on the same sampling time (P-value < 0.05).
WBC and platelet counts were also slightly but significantly decreased, and became normal after simmondsin-treatment was stopped.

LDH and bilirubin concentrations did not change with simmondsin treatment (data not shown).

Blood smears revealed normal blood cell morphology, and the reticulocyte count remained normal (data not shown).

The bone marrow smears showed very active blood cell formation but no differences were observed between the treatment groups. However, more megaloblastic cells were observed in the simmondsin groups than in the other groups.

No abnormal iron load was observed in liver, kidneys and spleen pathological examination.

An increase in relative organ weights (weight/body weight) was observed for the heart, lungs, liver, kidney, spleen and pancreas. After re-feeding, this increase normalized almost immediately in all organs, except for the kidneys which remained somewhat heavier. The weight of the thymus did not differ between the treatment groups.

At the microscopic level, no abnormalities were observed in the organs studied, except for the mesenteric lymph nodes found in the vicinity of the cecum. These lymph nodes were reddened and clearly visible with the naked eye. At microscopic examination, their peripheral sinus was filled with RBCs and there was active erythrophagocytosis. After withdrawal of simmondsin the red cells disappeared from the sinus, leaving a brownish iron pigment called hemosiderin. These hemolymph nodes have been previously described by the research group (Cokelaere et al., 1993). Their appearance is consistent with vasodilatation. The origin of this dilatation remains to be elucidated.

The biochemical parameters for liver and kidney function remained normal. These parameters were also found to be normal in previous experiments (Cokelaere et al., 1992b).

The lack of pathological changes at both the microscopic and biochemical level, taken together with the clear vasodilatation observed in the mesenteric lymph nodes, suggest that the organ weight changes might reflect a higher blood volume, due to dilatation of the blood vessels.

All the blood parameters indicate that simmondsin treatment causes a macrocytic normochromic anemia, as an increase is seen in MCV or red cell volume but a normal MCHC. The lowered WBC and platelet count have also been described in this form of anemia. This form of anemia can be caused by a great number of factors, such as a reduced intestinal production or absorption of vitamin B12 or of folic acid, or a disturbance in the metabolism of these factors. Vitamin B12 and folic acid play a role in DNA synthesis, so when they are lacking, cell division in fast dividing tissues may be inhibited, especially in the blood cell forming bone marrow. In those cases, cell nucleus division, before RBCs are delivered from the bone marrow into the bloodstream, is abnormal (Hillman, 1971). Thus the number of RBCs decreases, but the hemoglobin synthesis remains normal. This results in bigger RBCs with a normal hemoglobin concentration.

4. General conclusions

Simmondsin with a purity of over 99%, mixed in the food at 0.25% for 20 weeks in young growing male Wistar rats, induced a slight but statistically significant macrocytic, normochromic anemia that recovered completely after re-feeding. The internal organs became relatively heavier than in pair-fed animals but regained their normal weight immediately after withdrawal of simmondsin, except for the kidneys, which remained somewhat heavier. No microscopic or biochemical signs of toxicity were observed.

Acknowledgements

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