Cardiotoxicity of *Senna occidentalis* in sheep (*Ovis aries*)

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**Abstract**

The cardiotoxicity of Coffee senna (*Senna occidentalis*) was investigated in sheep that were fed diets containing its seeds, which are recognized as the most poisonous part of such weed. Dianthrone, the main toxic component of *S. occidentalis*, is known to impair mitochondrial oxidative phosphorylation, leading to myofiber degeneration. In this study, fifteen ewes were fed 0%, 2%, or 4% of seeds of *S. occidentalis* for 63 days. Non-specific markers of myocyte injury and electrocardiograms were undertaken at baseline, and at 14, 35, and 63 days after the animals were first fed the diets, while histopathology of heart samples was performed at the very end of the study. Our results showed an increase in serum AST and LDH over time, while CK-MB did not change significantly. Changes that could be ascribed to myocardial damage were not documented in the electrocardiograms. Cardiac histopathology demonstrated only mild-to-moderate vacuolar degeneration, myofiber edema and disarray, structural disorganization, and cellular necrosis. In conclusion, *S. occidentalis* caused myocardial fiber degeneration in a dose-dependent fashion, but the electrocardiogram was not able to identify these lesions non-invasively. Because the markers of myofiber injury used in this study lack specificity, they may not be used to support cardiac impairment objectively, despite some of them did change over time.

**Keywords:** Coffee senna, Electrocardiogram, Myofiber disarray, Plant toxicity.

**Introduction**

*Senna occidentalis*, a weed belonging to the family *Caesalpinoideae*, is recognized as one of the most important poisonous plants in veterinary medicine (Tasaka et al., 2000; Górniak, 2008). This leguminous plant is commonly found in summer crops, therefore having its seeds contaminate the harvest, which will later be consumed together with the feed (Riet-Correa et al., 1998; Haraguchi et al., 2003). Poisoning can occur when all the plant is ingested, but most commonly it occurs indirectly when its seeds are not separated from cereal grains. Therefore, it is a concern even for animals under intensive confinement (Górniak, 2008). Although all parts of *S. occidentalis* are poisonous, the seeds are thought to be more toxic (Haraguchi et al., 1998) because of the elevated concentrations of dianthrone, an anthraquinone that interferes with the function of mitochondria, leading to swelling and impairment of its inner structure (Barbosa-Ferreira et al., 2005; Górniak, 2008). Either natural or experimental ingestion of *S. occidentalis* have been investigated in several species and resulted in an afebrile disease, which is characterized by prostration, muscle twitches, diarrhea, myoglobinuria, motor incoordination, and death (Barbosa-Ferreira et al., 2005).

Skeletal muscle degeneration has been reported as a common finding in necropsies of several animal species. Alterations in the liver, central nervous system, and heart muscle have been documented as well (Tokarnia et al., 2002). Also, skeletal muscle histopathology demonstrated myofiber atrophy and interstitial edema, while vacuolar degeneration with structural disarray has been recognized in myocardial samples (Górniak, 2008).

To the best of the authors’ knowledge, the cardiotoxicity of *S. occidentalis* has never been investigated in ovines fed a diet containing increasing levels of dianthrone. Also, the potential use of electrocardiography as a non-invasive surrogate for myocardial injury was certainly not studied in animals being fed *S. occidentalis*. In this study, we hypothesized that diets containing more seeds of this weed would result in more severe cardiac lesions. Therefore, the purpose of this investigation was threefold: 1) to investigate how the serum levels of non-specific markers of myofiber injury is affected in sheep fed *S. occidentalis*; 2) to determine whether electrocardiography could potentially identify any cardiac lesion caused by the diet; and finally, 3) to use histopathology to assess the changes in cardiac structure.

**Material and Methods**

**Animals**

Fifteen mature mixed-breed ewes were recruited into a prospective experimental study. Inclusion criteria included the animals being completely healthy, as determined by a detailed clinical examination and

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while the positive electrode was positioned just behind the left olecranon on the left chest wall (over the apex beat area of the heart) (Fig. 1). Several ECG parameters were measured, including P wave duration ($P_{ms}$), P wave amplitude ($P_{mV}$), duration of PR interval ($PR$), duration of QRS complex ($QRS$), R wave amplitude ($R$), S wave amplitude ($S$), duration of QT interval ($QT$), T wave amplitude ($T_{mV}$), and duration of RR interval ($RR$). Changes in cardiac rhythm were also recorded.

**Histopathology of the heart**

A total of five cardiac tissue samples, which included the left ventricle free wall, right ventricle free wall, left atrium, right atrium, and the interventricular septum, were harvested from each animal. Samples were immediately fixed in 10% neutral-buffered formalin for 24 hours, and the fixed material was stored in 70% ethanol prior to processing into paraffin. Later, the samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin staining. All heart samples were semi-quantitatively scored for injuries on a scale of 0 (absent), 1 (mild, small focal lesions), 2 (moderate, multifocal or focally extensive lesions), and 3 (severe, affecting most areas). A positive result was considered when at least one sample was altered in a given animal.

**Statistical analysis**

The enzyme and electrocardiographic data was tested for normality using the Shapiro-Wilk test. Either a repeated measures analysis of variance or the Friedman test was used to check for differences in these parameters over 63 days of study. Chi-square test was performed to look for an association between diet and the identification of myocardial lesions on histopathology. The software Prism for Windows v. 5.04 (Graphpad Software, San Diego CA, USA) was used for all statistical analyses, and probability < 0.05 was considered significant.
Results

Table 1 shows the means and standard deviations of the plasma enzymes in all groups. AST and LDH increased significantly over time when diets containing either 2% or 4% of *S. occidentalis* seeds were fed to the animals. No significant alteration was documented for CK-MB data undergoing an analysis of variance. The electrocardiographic data is listed in Table 2, which shows an absence of consistent changes along the experimental period. Only sinus rhythm and sinus arrhythmia were identified throughout the study (Fig. 2). We observed variations in rhythms and sinus arrhythmia became more prevalent over time in ewes being given diets with either 2% or 4% *S. occidentalis* seeds. Nevertheless, an association between diets and rhythms could not be demonstrated by chi-square tests (0% \(P=0.6444\); 2% \(P=0.1718\); 4% \(P=0.2795\)).

![Fig. 2. Distribution of heart rhythms in sheep that were fed diets containing 0%, 2% or 4% of *Senna occidentalis* seeds. Only sinus rhythm (SR) and sinus arrhythmia (SA) were documented over time and no significant association could be demonstrated between diets and heart rhythms.](image1)

![Fig. 3. Representative findings of the histopathological assessment of the left ventricular free wall from ewes fed a diet containing 0% (A), 2% (B) or 4% (C) of *Senna occidentalis* seeds. (A) Normal cardiac myofibers exhibiting a completely normal structural organization; (B) enlarged nuclei are seen within the myofibers; (C) enlarged nuclei and vacuolation of the cytoplasm around the nuclei are shown (H&E, 40x).](image2)

Table 1. Serum enzymes [mean (SD)] measured in ewes fed a diet containing 0%, 2% or 4% of *Senna occidentalis* seeds.

| Diet (%) | Baseline | 14 days | 35 days | 63 days |
|----------|----------|---------|---------|---------|
| AST (U/L) |          |         |         |         |
| 0        | 101.2 (14.9) | 105.0 (24.4) | 123.0 (9.9) | 127.7 (25.5) |
| 2        | 92.6 (16.6) | 98.9 (6.8) | 113.1 (4.9) | 160.7 (48.4)* |
| 4        | 77.5 (18.0) | 123.0 (25.0) | 125.4 (24.3) | 149.3 (50.7)* |
| CK-MB (U/L) |          |         |         |         |
| 0        | 101.7 (96.7) | 110.1 (43.9) | 135.0 (75.5) | 145.0 (45.8) |
| 2        | 200.0 (44.1) | 160.0 (41.0) | 258.3 (100.2) | 185.8 (75.7) |
| 4        | 200.0 (67.1) | 143.3 (44.5) | 188.3 (54.2) | 128.8 (45.6) |
| LDH (U/L) |          |         |         |         |
| 0        | 817.2 (165.4) | 666.4 (88.1) | 710.4 (140.4) | 1023.0 (273.8) |
| 2        | 276.6 (276.6) | 715.2 (220.0) | 823.2 (41.5) | 1088.7 (506.5)* |
| 4        | 113.4 (113.4) | 815.6 (152.3)* | 927.2 (145.6)* | 886.6 (240.3)* |

*Significantly different (\(P<0.05\)) from the baseline measurement at the *post hoc* test. AST: Aspartate aminotransferase, CK-MB: Creatine kinase MB fraction, LDH: Lactate dehydrogenase
On histopathological examination the microscopic structure of the heart of animals that were fed diets with no *S. occidentalis* appeared normal (Fig. 3A). On the contrary, all animals that were fed diets containing *S. occidentalis* seeds had lesions detected in at least one cardiac sample. When a diet containing 2% of seeds was fed to the ewes, 36% (9/25) of the samples exhibited any abnormality (8/25 mild; 1/25 moderate), which included

### Table 2
Results [mean (SD)] of the base-apex electrocardiograms recorded in ewes fed a diet containing 0%, 2% or 4% of *Senna occidentalis* seeds.

| Diet (%) | Baseline | 14 days | 35 days | 63 days |
|----------|----------|---------|---------|---------|
| P<sub>ms</sub>| 52.60 (4.32) | 50.60 (5.75) | 60.80 (10.91) | 51.40 (3.38) |
| 2        | 58.80 (8.35) | 63.20 (6.43) | 62.50 (4.82) | 63.20 (8.73) |
| 4        | 52.80 (3.43) | 57.80 (6.68) | 59.40 (6.71) | 57.20 (6.27) |
| P<sub>m</sub>V| 0.19 (0.03) | 0.12 (0.03)* | 0.14 (0.02) | 0.16 (0.03) |
| 2        | 0.15 (0.05) | 0.11 (0.03) | 0.14 (0.03) | 0.17 (0.01) |
| 4        | 0.15 (0.04) | 0.13 (0.02) | 0.13 (0.03) | 0.17 (0.05) |
| PR       | 97.00 (8.00) | 95.20 (10.21) | 99.40 (16.28) | 92.80 (11.30) |
| 2        | 112.00 (12.93) | 118.60 (8.43) | 105.25 (11.14) | 112.25 (7.19) |
| 4        | 98.20 (10.54) | 107.80 (10.01) | 105.80 (21.90) | 101.20 (13.48) |
| QRS      | 70.60 (9.85) | 53.20 (6.71)* | 65.20 (9.20) | 63.20 (7.81) |
| 2        | 56.00 (2.68) | 56.00 (5.25) | 66.75 (15.22) | 57.50 (7.63) |
| 4        | 63.00 (11.02) | 57.20 (5.81) | 57.20 (8.21) | 60.80 (7.14) |
| R        | 0.03 (0.01) | 0.02 (0.004) | 0.03 (0.01) | 0.03 (0.01) |
| 2        | 0.07 (0.06) | 0.06 (0.08) | 0.05 (0.04) | 0.06 (0.04) |
| 4        | 0.03 (0.01) | 0.03 (0.01) | 0.02 (0.005) | 0.03 (0.01) |
| S        | 0.67 (0.26) | 0.34 (0.12) | 0.44 (0.19) | 0.48 (0.06) |
| 2        | 0.47 (0.17) | 0.31 (0.13) | 0.40 (0.12) | 0.49 (0.16) |
| 4        | 0.54 (0.19) | 0.37 (0.12) | 0.43 (0.10) | 0.60 (0.25) |
| QT       | 279.40 (28.16) | 288.80 (20.25) | 269.40 (28.09) | 280.80 (25.51) |
| 2        | 279.20 (18.51) | 304.60 (38.53) | 265.75 (24.77) | 256.75 (46.49) |
| 4        | 294.60 (14.29) | 311.80 (36.19) | 263.80 (20.57) | 284.60 (34.00) |
| T<sub>m</sub>V| 0.38 (0.15) | 0.18 (0.09) | 0.25 (0.11) | 0.38 (0.21) |
| 2        | 0.13 (0.10) | 0.17 (0.15) | 0.19 (0.13) | 0.29 (0.05) |
| 4        | 0.18 (0.06) | 0.12 (0.03) | 0.18 (0.09) | 0.33 (0.14) |
| RR       | 527.20 (128.11) | 625.60 (86.90) | 512.60 (112.14) | 524.20 (116.21) |
| 2        | 646.80 (59.64) | 692.80 (140.80) | 464.75 (49.06)* | 525.75 (85.73) |
| 4        | 655.60 (99.16) | 713.00 (198.93) | 502.40 (127.27) | 553.40 (59.56) |

*Significantly different (P<0.05) from the baseline measurement at the post hoc test. P<sub>ms</sub>: P wave duration, P<sub>m</sub>V: P wave amplitude, PR: Duration of PR interval, QRS: Duration of QRS complex, R: Amplitude of R wave, S: Amplitude of S wave, QT: Duration of QT interval, T<sub>m</sub>V: Amplitude of T wave, RR: Duration of RR interval.

On histopathological examination the microscopic structure of the heart of animals that were fed diets with no *S. occidentalis* appeared normal (Fig. 3A). On the contrary, all animals that were fed diets containing *S. occidentalis* seeds had lesions detected in at least one cardiac sample. When a diet containing 2% of seeds was fed to the ewes, 36% (9/25) of the samples exhibited any abnormality (8/25 mild; 1/25 moderate), which included
In this study we sought to investigate the effects of diets containing increasing concentrations of *Senna occidentalis* seeds, which are recognized as the most poisonous part of that weed. Our main goals were to determine whether diets containing varying concentration of the plant seeds would produce changes, over a two-month period that could be documented by the electrocardiogram, by the serum concentration of some non-specific markers of cardiac injury, and by histopathology of the heart. AST and LDH serum concentrations increased significantly over time (Table 1). However, the non-specific nature of these enzymes, which may be released under a wide array of injuries, including liver damage, preclude its sole use to identify cardiac impairment specifically.

As mentioned before, the ECG disclosed no significant changes when the ewes were fed the diets. The very few changes found on the electrocardiographic data occurred in animals receiving diets with no seed at all (Table 2), except for a reduction in RR interval observed at 35 days in animals fed 2% of seeds. The RR interval is inversely related to heart rate, therefore indicating an increased heart rate at the same moment. It is worth mentioning, however, that the instantaneous heart rate recorded by the electrocardiogram is heavily influenced by environmental conditions, including those that potentially cause stress to the animals, such as the physical restraint needed for ECG recording (Lago et al., 2009). In a study in which 31 healthy ovinas were enrolled, Schultz et al. (1972) found the heart rate to range from 60 to 197 bpm, showing its strong variation. Later, another investigation also found an increased mean heart rate of 119 bpm in normal sheep (Tório et al., 1997). In this study, however, the mean heart rates remained within the normal range for ovinas at all times. Sinus rhythm was the most prevalent cardiac rhythm at baseline evaluation, which is consistent with prior studies in sheep (Tório et al., 1997; Mir et al., 2000; Lago et al., 2009). However, when diets containing seeds were given to the animals, there was a progressive non-significant change to sinus arrhythmia, which became the most prevalent rhythm at 63 days animals fed either 2% or 4% of *S. occidentalis* seeds. Curiously, sinus arrhythmia is not considered a malignant arrhythmia, since it results from variations in autonomic balance. Therefore, increases in sympathetic tone accelerate the heart rate, while the attenuated sympathetic tone reduces the heart rate. To the authors’ knowledge, however, the effects of *S. occidentalis* in the autonomic balance was never investigated, and at this time we might speculate that these findings could be ascribed to the animals being more used to the exam room, therefore having an attenuated sympathetic response along the procedure.

In contrast, the microscopic assessment of heart samples showed varying degrees of structural damage to the myocardial cells, which was more prevalent and severe in animals being given a 4%-seed diet. This condition is likely supportive of the toxicity of *Senna occidentalis* on the heart occurring in a dose-dependent fashion. Interestingly, no changes in QT interval could be observed, suggesting no impairment in ventricular total electrical activity in spite of the structural damage and myofiber disarray. P waves, which represent the atrial electrical activity, were consistently documented in all animals regardless of the diet being fed to them. Prior studies in ovinas indicated a positive P wave in the majority of leads (Schultz et al., 1972; Tório et al., 1997), which is similar to our findings. In this study, 100% of the electrocardiograms exhibited positive P wave at lead II. It is likely that myocyte disorientation and myofibrilar disarray were not able to create an electrical milieu and substrate for supraventricular arrhythmias in these animals.

Alterations in cardiac muscle have been documented in several species being given *S. occidentalis*. Necrosis, vacuolar degeneration, myofiber edema, and structural disorganization (Fig. 3B). For the animals receiving diets with 4% of seeds, 52% (13/25) of the samples had alterations (9/25 mild; 4/25 moderate), including a complete structural disorganization, with myofiber disarray, small, dark-staining pyknotic nuclei, and acidophilic cytoplasm, which are indicative of cellular necrosis (Fig. 3C). Although Chi-square test found a significant ($P=0.0002$) association to exist between diets and the identification of myocardial lesions on histopathology (Fig. 4), severe lesions affecting most areas were not documented in any animal enrolled in this investigation.

**Discussion**

Alterations in cardiac muscle have been documented in several species being given *S. occidentalis*. Necrosis, vacuolar degeneration, myofiber thinning, swelling and replacement by connective tissue are all

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**Fig. 4.** Identification of myocardial injuries on histopathology in sheep that were fed diets containing 0%, 2% or 4% of *Senna occidentalis* seeds. A significant association ($P=0.0002$) was found to exist between the increasing amount of seeds in diets and the documentation of any cardiac lesions, which included cell vacuolation, myofiber swelling and disarray, pyknotic nuclei, acidophilic cytoplasms, and varying degrees of structural disorganization.
reported in many preceding investigations (O’Hara et al., 1969; Martins et al., 1986; Barros et al., 1990; Tasaka et al., 2000). Dianthrone, the main toxic component of *S. occidentalis*, is known to induce the uncoupling of mitochondrial oxidative phosphorylation, which is absolutely necessary to produce energy for the constant pumping action of the heart. Such energy-deprivation impairs the sodium-potassium pump activity and, as a result, water accumulates within the cell leading to myofiber degeneration (Barros et al., 1990). Similarly, feeding diets containing either 2% or 4% *S. occidentalis* seeds to animals in this study for 63 days was enough to produce myocardial lesions that included vacuolation, pyknotic nuclei, acidophilic cytoplasms, and swelling of myofiber bundles, which resulted in varying degrees of structural disarray that are similar to previous reports (Suliman et al., 1982; Suliman and Shommein, 1986; Barros et al., 1999).

Among the several limitations of this investigation are the small number of animals recruited, the relatively short period of time during which the animals were fed the seed-containing diets, the absence of a cardia-specic biomarkers such as troponin I, and finally, the absence of an echocardiographic assessment of the hearts to evaluate remodeling and function *in vivo*. Also, a more detailed microscopic assessment of the heart samples could reveal information on remodeling of the extracellular matrix, and whether apoptosis also played a role in the degenerative process ascribed to *S. occidentalis*.

**Conclusion**

In conclusion, diets containing either 2% or 4% of *Senna occidentalis* seeds caused myocardial fiber degeneration in ewes in a dose-dependent fashion. A wide array of lesions was observed, but all animals had at least one lesion documented on cardiac histopathology. Although only mild lesions could be documented, this study found that a few animals that were given diets containing the plant seeds developed lesion within the myocardial tissue. Serum AST and LDH increased over time and might potentially aid in detecting myofiber injury. On the contrary, the electrocardiogram was not able to document any changes that could be related to myocardial damage.

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