Hematological, biochemical and histological evaluation of sub-chronic administration of ethanol leaf extract of *Canscora decussate* on Kidney and Stomach of wistar rats

1Joseph Opeyemi Tosin, 1*Joseph Oyepata Simeon.

1Department of Pharmacology, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria.

1Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University, Oye–Ekiti, Ekiti State, Nigeria.

Abstract

*Canscora decussate* is traditionally believe to relieve renal and gastro intestinal tract disorder. Ethanol leaf extract of *Canscora decussate* was tested for chronic effect on Kidney and Stomach of wistar rats by evaluating the hematological, biochemical and histological parameters of these organs. The study was done by grouping the animals in to 4 groups with each group receiving different concentrations of the extract (100-400mg/kg) respectively for 28 days, physical observation of the rats was carried out every day of the study. Mean corpuscular volume, and red blood cell levels decreased significantly (P≤0.05) at 200 and 400 mg/kg doses, but not basophiles, neutrophils, eosinophils, or platelets. There was no significant change (p≤0.05) in serum sodium chloride, potassium creatinine and urea when compared to the control group at all doses delivered. Slight lymphocyte hyperplasia with normal kidney histology architecture observed at all doses and the control (10 ml/kg). *Canscora decussateis* relatively safe for use as it does not possess danger against traditional use.

Key words: *Canscora decussate*, blood, rats, kidney, stomach

*Correspondence: Joseph Oyepata Simeon*,

Email Address: oyepata.joseph@fuoye.edu.ng Telephone No.: +2348038248352

Introduction

Each kidney is believed to be about the size of a fist [1]. The kidneys filter excess water and waste from the blood while creating urine. Renal disease impairs the kidneys' ability to adequately filter blood. Diabetes and hypertension both raise the risk of kidney injury [2]. Dialysis or a kidney transplant can be used to treat renal failure. Other kidney problems include infections, cysts, stones, and acute renal injury [2]. To keep the body healthy, strong kidneys are essential. They are in charge of eliminating waste, excess water, and other contaminants from the bloodstream. Toxins are accumulated in the bladder and are discharged during urination [4].
The kidneys help to maintain the pH, salt, and potassium levels in the body [4]. They produce hormones that regulate blood pressure and the production of red blood cells. The kidneys also stimulate the production of a kind of vitamin D that aids calcium absorption in the body. Malnutrition, brittle bones, nerve damage, and other health issues can result from kidney disease [5].

Mescher [6] defines the stomach as a muscular, hollow organ found in the gastrointestinal systems of humans and many other animals, including a number of invertebrates. With its dilated appearance, the stomach is an important organ in the digestive system. The stomach enters the gastric phase of digestion after chewing. To achieve a chemical breakdown, enzymes and hydrochloric acid are used. In humans and many other animals, the stomach is located between the esophagus and the small intestine. The stomach secretes digestive enzymes and gastric acid to aid in food digestion. The pyloric sphincter controls the passage of partially digested food (chyme) from the stomach into the duodenum, where it is peristaltically transported through the intestines [7].

*Canscora decussate* is cultivated in the gardens as ornamental plant for its flowers. This is an annual herb with 4-winged stem and half a meter in length with decussate branches. It grows well in moist conditions. Leaves are sessile, 2.5-4 cm in length, lanceolate, decussate with 3 prominent vertical lines; flowers are axillary, solitary, and white or yellowish in color. The entire plant, as well as fresh juice, is used in the traditional medicine for the treatment of insanity, epilepsy, and nervous debility [8]. This plant contains bitter substances and an oleoresin. It is also found to contain triterpines, alkaloids, and xanthones [8]. It is also a natural source of pentaoxygenated, hexaoxygenated, and dimeric xanthones [9]. It is used in cerebral abnormalities, epilepsy, insomnia, burning sensation, kidney disease, GIT disorder, edema, urinary disorders, snake-bites, and diseases caused by evil spirits. It is recommended as tonic for brain and nerves conditions and was also for sexual and seminal debilities [10-11].

The aim of this study was to evaluate the hematological, biochemical and histological effect of sub-chronic administration of ethanol leaf extract of *Canscora decussate* on kidney and stomach of Wistar rats.

**Material and Methods**

**Study area**

This study was conducted at the University of Uyo, Akwa Ibom State, Nigeria. Between March 11, 2022 and April 29, 2022. Uyo, Nigeria is in the Nigerian National Urban Areas category with GPS coordinates of 5° 2’ 20.2668” N and 7° 54’ 34.0920” E on the day of arrival. Uyo has a summer season, a rainy season, and a hot, dry climate; Annual temperatures range from 69°F to 87°F. [12] (Fig. 1).
Figure 1. Map of the research area. [12]

**Plant collection**

*Canscora decussate* was acquired at a conservative medicinal garden in Uyo, Akwa Ibom State, Nigeria. The plant was verified by the Department of Botany at the University of Uyo in Akwa Ibom State, Nigeria. The voucher specimens were stored in the herbarium department (No: 276).

**Animals**

Male Wistar rats were obtained from the University of Uyo Animal House. They were fed animal pellets purchased from Grand Cereals Limited and given unlimited water. Permission and approval for the animal study was granted by the Animal Ethics Committee of the Faculty of Health, University of Uyo (UU/2022/115). Rats (n = 6) were placed in different treatment groups. Animal studies were conducted under similar conditions. Care and handling of animals in accordance with public health guidelines in the Guide for the Care and Use of Animals (2011).

**Plant extraction**

For two weeks, the leaves were shade dried. The plant leaf material was further broken down and pulverized into fine particles. The ethanol extract of *Canscora decussate* was prepared using 429g of the powdered leaves mixed in 1287 ml (1:3 w/v) of 70% ethanol for 72 hours while it was stirred intermittently. The combined extract was filtered through a vacuum filter using Whatman No.1 filter paper. The resulting solution was evaporated using water bath heater at 55°C to yield 52.1g of the crude
extract corresponding to a percentage yield of 18%. The extract was stored at 4°C until required.

**Dosage selection**

The LD$_{50}$ of the plant had been previously established to be ≥ 5000 mg/kg [14]. Dosage of extract administered to animals was determined from 1000 mg.kg$^{-1}$, 500 mg/kg and 250 mg/kg of the estimated LD$_{50}$ as described by Neharkar and Galkwad [16].

**Animal study**

The protocol for this work was in accordance with that of Sabastine et al. [9] Twenty-four rats (weighing 182-256g) of male rats were chosen at random and divided into four groups of six rats each. Groups 2, 3, and 4 received 100, 200, and 400 mg/kg of *Canscora decussate* extract, respectively, while group 1 served as the control group, receiving normal saline (10ml/kg). The weights of the rats were recorded at the start of the experiment and once a week thereafter.

**Sample collection**

After 28 days the animals were only provided with water for the next twenty-four hours after which they were euthanized using diethylether. A heart puncture was used to collect blood samples. Blood samples were obtained in EDTA-containing sample bottles for hematological analysis and in plain bottles for biochemical analysis. Allowing the blood in the plain container to coagulate, it was centrifuged at 3500rpm for 10 minutes before being split into labelled containers.

**Qualitative phytochemical screening**

The test was carried out according to the procedures outlined by Trease and Evans [12] and Harbourne [11]. Ten percent (10%) preparation of the extract in distilled water was considered as the test samples. Distilled water was used as a negative control throughout the phytochemical tests.

**Kidney Function Test**

Electrolytes (Na$^+$, K$^+$, Cl$^-$, and HCO$_3^-$), creatinine, and blood urea levels were tested using diagnostic kits (ELI Tech, Puteaux, France) as indications of renal function. The aforementioned characteristics were determined at the University of Uyo Teaching Hospital’s Chemical Pathology Department. Kidneys were removed and stored in 10% formal saline solution before being processed, sectioned, and stained with hematoxylin and eosin (H&E) according to standard procedures.

**Histological study**

Kidney and stomach were removed and stored in buffered formalin for 12 h before histological examination. Histopathological examination was performed using a light microscope. A histopathologist was blind to the coded treatment group and reviewed the slides. Hematoxylin and eosin staining technique was used in this study.

**Data analysis**
The data were expressed using the mean and standard error of the mean (SEM). Before Dunnett's post hoc test for multiple comparisons between the control and treated groups, one-way ANOVA was performed to statistically examine the data. P≤0.05 was considered significant.

Results

Phytochemical analysis of *Canscora decussate*

Phytochemical test was carried out on the whole ethanol extract as well as the. The results are shown in Table 1. Phytochemical screening of all the crude extract of *Canscora decussate* extract showed the presence of various chemical constitutions mostly Alkaloid, flavonoid, cardiac glycoside, saponins, tannins, terpenes, reducing sugar, carbohydrate

Effect of *Canscora decussate* on relative organ to body weight ratio in rats.

Rats receiving 200 mg/kg of the ethanol extract of *Canscora decussate* were found to have slightly larger value kidney and stomachs when compared to the control group. There was no significant (p<0.05) difference at 100 and 400 mg/kg dose (Table 2).

Effect of *Canscora decussate* on hematological parameters in wistar rats.

When compared to the control group, there was a significant (p≤0.05) decrease in HGB, MCV, and RBC at 100 and 200 mg/kg doses, but an improvement of the parameters at 400 mg/kg dose. The extract had no significant (p≤0.05) effect on the levels of Basophils and Eosinophils (Table 3).

Effect of *Canscora decussate* on kidney function

Result revealed *Canscora decussate* did not cause significant change (P ≤0.05) in serum sodium chloride, potassium creatinine and urea when compared to the control group at all doses delivered. There was significant change (P ≤0.05) in the level of serum sodium when compared to control group (Table 4 and figure 3).

Effect of leaf extract of *Canscora decussate* on histology of rat

There was no alteration in the histological integrity of the structure of the rat's kidney and stomach. Histological characteristics remained unchanged at all doses when compared to the control (Figure 4 and 5).

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical</th>
<th>Crude extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Reducing Sugars</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical Analysis of ethanol fruit extract of *Canscora decussate*
4 Flavonoids +++
5 cardiac Glycosides ++
7 Saponins +++
8 reducing sugar +
9 Terpene ++
10 cardiac glycoside ++
11 Acidic compounds –
12 carbohydrate ++

Table 2: Effect of 28 days administration of ethanol leaf extract *Canscora decussate* on relative organ to body weight ratio in rats.

<table>
<thead>
<tr>
<th>Relative Organ To Body weight</th>
<th>Treatment(mg/kg))</th>
<th>STOMACH</th>
<th>KIDNEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW(10 ml/kg)</td>
<td>0.87±0.0</td>
<td>0.55±0.14</td>
</tr>
<tr>
<td></td>
<td>100 mg/kg</td>
<td>0.85±0.42</td>
<td>0.51±0.36</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>0.92±0.32</td>
<td>0.69±0.31*</td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.97±0.11</td>
<td>0.60±0.91</td>
</tr>
</tbody>
</table>

*Significantly different from the distilled water (DW) control at p<0.05.

CD=*Canscora decussate*
### Table 3: Effect of ethanol leaf extract of *Canscora decussate* on hematological parameters in wistar rats.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Treatment (mg/kg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (10ml/kg)</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>9.27±0.72</td>
<td>7.75±1.12</td>
<td>4.11±1.02*</td>
<td>8.25±1.04</td>
</tr>
<tr>
<td>RBC (×10^12/L)</td>
<td>9.31±0.15</td>
<td>7.64±0.64</td>
<td>7.13±0.00*</td>
<td>8.32±0.54</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>16.23±0.67</td>
<td>16.23±0.11</td>
<td>12.53±0.12*</td>
<td>15.18±0.25</td>
</tr>
<tr>
<td>HCT (g/dL)</td>
<td>56.17±2.00</td>
<td>57.00±0.43</td>
<td>35.60±3.11*</td>
<td>54.00±1.02</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>67.33±1.00</td>
<td>66.04±1.43</td>
<td>58.17±0.24*</td>
<td>70.60±0.65</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.26±0.10</td>
<td>18.82±1.12</td>
<td>19.85±0.77</td>
<td>19.92±0.12</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>39.45±0.18</td>
<td>27.53±1.23</td>
<td>32.55±0.90*</td>
<td>27.11±0.97</td>
</tr>
<tr>
<td>PLT (×10^9/L)</td>
<td>630.85±12.00</td>
<td>567.11±13.403</td>
<td>568.10±18.19*</td>
<td>670.32±12.44*</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>96.82±4.12</td>
<td>85.13±4.16</td>
<td>82.11±5.03</td>
<td>89.44±3.17</td>
</tr>
<tr>
<td>NEUT (×10^9/L)</td>
<td>11.81±3.67</td>
<td>10.80±3.68</td>
<td>15.41±2.43</td>
<td>13.32±3.46</td>
</tr>
<tr>
<td>EOSI (×10^9/L)</td>
<td>1.11±0.11</td>
<td>1.40±0.11</td>
<td>1.53±0.52</td>
<td>1.28±0.12</td>
</tr>
<tr>
<td>BASO (×10^9/L)</td>
<td>1.91±0.20</td>
<td>2.10±0.13</td>
<td>2.32±1.14</td>
<td>3.02±3.11</td>
</tr>
</tbody>
</table>

*significantly different from the distilled water (CD = *Canscora decussate*, WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

### Table 4: Effects of *Canscora decussate* extract on kidney function
<table>
<thead>
<tr>
<th>Renal indices and electrolytes</th>
<th>Treatment (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW(10ml/kg)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>8.31±0.54</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>153.00±2.39</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>123.12±5.25</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>10.44±0.27</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>83.13±5.61</td>
</tr>
</tbody>
</table>

*Significant difference from control.
Figure 3: Effects of 28 days oral administration of *Canscora decussate* extract on kidney function
Figure 4: figure of the kidney (Hematoxylin and eosin. H and E x100). (1) Control group, Shows normal neurons (). (2) 100 mg/kg. (3) 200 mg/kg (4) 400 mg/kg of ethanol leaf extract of Canscora decussate
Figure 5. figure of the stomach (Hematoxylin and eosin. H and E ×100). (A) Control group, Shows. (B) 100 mg/kg. (3) 200 mg/kg (4) 400 mg/kg of ethanol leaf extract of *Canscora decussate*

**Discussion**

The plant *Canscora decussate* was studied to observe its effects on the kidney and stomach of wistar rats. *Canscora decussate* caused a significant decrease in red blood cell, hemoglobin, and platelet counts when compared to the control group of rats, indicating that the plant may limit red blood cell production, shorten red blood cell lifetime, or interfere with how the body uses iron. Anemia, or a decreased red blood cell count, can cause exhaustion and weakness. Maton *et al* [13,14]. Low RBC counts can create a number of symptoms and health concerns because they force the body to work harder to give enough oxygen to the cells [15]. The function of hemoglobin is to transport oxygen from the lungs to the blood and body. Myoglobin is a protein that accepts, transports, stores, and releases oxygen in muscle cells [16]. There was no effect of the extract on the number of basophils, neutrophils, lymphocytes, or eosinophils. This suggests that the plant has no effect on the body’s immune system. It could also mean that the plant has no ability to influence the immune system. In the platelet count, it was observed that there was significant reduction at 200 mg/kg and significant increase in it level at 4000 mg/kg. This is indicative that the extract may have a biphasic effect. That is, at certain dose it possesses certain characteristic while at a much higher dose the observed effect may be reversed. The haematological result suggests that at higher dose the plant may not affect or
may even have beneficial haematological relevance.

Because serum levels of urea, uric acid, K\(^+\) Na\(^+\) Cl\(^-\), and HCO\(_3\) were not altered in the rats utilized in the current study who received ethanol leaf extract of *Canscora decussate*, the kidney’s function was not compromised. The study also revealed that *Canscora decussate* induced a slight increase in serum Na and creatinine levels even at the lowest dose administered. This could be because of the plant's damaging and oxidative activity, which appears to be higher than the antioxidant molecule at lower concentrations. Creatinine levels can rise in some kidney diseases due to a loss of creatinine excretory function, muscle damage, or taking an improper drug that interferes with the kidney’s normal function [17,18]. The degradation of tissue creatinine is the most common endogenous source of creatinine [19]. The group that received 100 mg/kg of the extract had considerably higher serum creatinine levels than the control group.

According to Sabastine *et al* [20], elevated blood creatinine levels have been associated to possible renal disease. Ghosal *et al* [21] proposed in a study that an increase in serum creatinine levels could signal potential injury to the kidney’s functioning nephrons. Renal failure was most likely a potential consequence when serum creatinine levels are higher than the usual value [22]. Creatinine content in serum is an excellent diagnostic for chronic renal disease diagnosis. Increased serum creatinine concentration, as indicated by Ghosal *et al.* [23] and Simeon *et al.* [24] has been regarded to be a measure of nephrotoxicity assessment. Furthermore, at high concentrations, presence of phytochemicals with antioxidant potentials in *Canscora decussate* maybe responsible for providing cells with defense capabilities. Because serum urea was unaffected in this investigation, it's probable that the plant only causes minor kidney injury. When compared to controls, histological analysis indicated minimal cellular changes in all groups after a 28-day administration of an ethanol extract of *Canscora decussate* to the kidney.

The renal and stomach architecture of the rat remained histologically intact. At all doses, histological characteristics remained constant when compared to the control. Previous study has shown that *Canscora decussate* contains proteins, carbohydrate flavonoids, glycosides, triterpene saponins, phenol, and anthraquinone flavonoids [25,26]. Saponins, tannins, phenols, and triterpenoids, which have antioxidant qualities, may have resulted in decreased tissue necrosis in the organs studied [27].

**Conclusion**

*Canscora decussate* is a plant with many different traditional uses around the world. Result from this study revealed consumption of *Canscora decussate* for a particular period may not have serious consequences on some vital body organs. More studies is needed to be carried out to determine sub-chronic and chronic consequences of consuming the plants.

**Conflict of Interest:** The authors declare that there are no potential conflicts of interest.
Acknowledgement

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