



Effective evaluation of poison in *Cryptolepis sanguinolenta* for use in veterinary medicine

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Abstract

Acute and sub-acute oral toxicity assessment of the aqueous root extract of *Cryptolepis sanguinolenta* was studied in Sprague Dawley rats for possible use as animal medication. The extract (250 - 3000 mg/kg, p.o) was administered daily for a period of 72 h and (500 - 2000 mg/kg, p.o) for 14 days for acute and sub-acute studies respectively. Acute administration of the extract did not produce any physiological and behavioural changes. In the subacute toxicity studies however, a dose-dependent increase in the number of platelets (from a vehicle-treated control value of $353.00 \pm 49.40 - 958.00 \pm 42.50$ in animals treated with 2000 mg/kg) was observed. Granulocyte number also increased dose-dependently ($0.77 \pm 0.15 - 3.70 \pm 0.20$) from the vehicle-treated control to the group that received 2000 mg/kg, indicating possible inflammation. Central nervous system toxicity and marginal enlargement of liver and kidney were evident in the 2000 mg/kg treated group. These findings however did not correlate with the biochemical and histopathological studies as no pathological changes occurred in the renal or hepato-biliary systems. The present results suggest that the aqueous root extract of *C. sanguinolenta* < 500 mg/kg orally is generally safe. However, caution should be taken with doses > 500 mg/kg as these may induce thrombocytosis, inflammation and central nervous system toxicity.

Keywords: Sub-acute toxicity, rats, *Cryptolepis sanguinolenta* extract

INTRODUCTION

Small ruminants contribute considerably to national food security, improved rural livelihoods and significant rural poverty reduction (Aryee et al., 1991). In Ghana for example, of the about 3.7 million goats and 3.1 million sheep presently, 90% are raised under the traditional extensive system by smallholder livestock farmers. Poor health is the most important constraint to small ruminant production under this system. Each year large numbers of farm animals are lost to disease. It is estimated, for example that, control of diarrhoea and parasitic disease in these animals could increase their production by about 60% (Aryee et al., 1991). Use of modern chemotherapeutic drugs is currently seen as the most effective means of controlling livestock diseases. In rural Ghana and other parts of the West African sub-region, orthodox veterinary drugs are not available in many places or are

expensive and out of reach of many livestock farmers. Many smallholder farmers therefore rely on much cheaper and available herbal medicines for keeping their animals healthy and productive.

Recently, there has been awareness on the use of herbs in animal health care practices (MacCorkle, 1989; Bizimana and Schrecke, 1995). The comparatively low cost of herbal medicines, their relative safety and their availability can impact positively on animal production. Elsewhere, in India for example, resource-poor farmers depend exclusively on plant medicines for the treatment of animal diseases (Paduakuma, 1998).

Cryptolepis sanguinolenta (Lindl.) (Periplocaceae) also known as Ghana quinine is basically used in traditional medicine for the management of malaria. It is found in

tropical forest clearings as it grows commonly in dispersed open areas (Lou et al., 1998). Dried *Cryptolepis* has a sweet fragrance (Irvine, 1961; Oliver-Bever, 1986; Sofowora, 1982; Dokosi, 1998). *C. sanguinolenta* is also traditionally known to possess cure for both bacterial respiratory and enteric diseases of animals and humans (Irvine, 1961; Ayensu, 1978; Dokosi, 1998). In Ghana, the dried root decoction of the herb prepared by boiling the powdered roots in water is used to treat various forms of fevers, urinary and upper respiratory tract infections, septicaemia or powdered as a castrant for wounds in animals and humans (Boakye-Yiadom, 1979; Boye et al., 1990; Wright et al., 1996). In spite of the massive indigenous knowledge on the ethno-veterinary uses of this plant, pharmacological and particularly toxicological studies that will assess the safety and promote it for animal use are limited (Mathias, 1996). In contributing to the search for safe and efficacious herbal medicines in animal health care, this paper reports the result of toxicological assessment of *C. sanguinolenta* for possible use in animals.

MATERIALS AND METHODS

Plant material and preparation of extracts

C. sanguinolenta roots were collected from Mampong-Akwapim, in the Eastern Region of Ghana. Identification and authentication was done by scientists at Centre for Scientific Research into Plant Medicine. Mampong - Akwapim, where the plant is routinely used in the treatment of malaria. The harvested roots were sun-dried for 3 - 5 days after which they were pulverized. One kilogram of the dried pulverized root was boiled under reflux condensation in 2 L of water for 1 h. The extract was filtered hot in a tincture press and allowed to cool. The filtrate was freeze-dried to yield 21.3 g (2.13%) of the brown substance that was stored in a desiccator until it is used. The freeze-dried extract was suspended in 2% tragacanth and administered to the animals as appropriate using a stomach canula.

Animals

Healthy adult Sprague Dawley (SD) rats (150 - 250 g) of both sexes obtained from Noguchi Memorial Institute of Medical Research (NMIMR) and maintained at the animal house of the Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana were used. They were kept in rat cages and fed a balanced pelleted diet obtained from Ghana Agricultural Foods Company Ltd (GAFCO) Tema, with free access to water. The studies were conducted in accordance with internationally accepted principles for laboratory animal use and care (EEC directive of 1986: 86/609 EEC) and were approved by the departmental ethics committee.

Acute toxicity

Five groups of SD rats ($n = 6$) received (250 – 3000 mg/kg p. o) of extracts of *C. sanguinolenta* suspended in 2% tragacanth. One group served as the control and received only 2% tragacanth. The animals were observed over a 24-hour period for autonomic and behavioral effects, and other signs of toxicity. After 24 hours three animals in each group were weighed and euthanized. Blood samples were obtained from the jugular vein for haematology and blood

chemistry. Internal organs (liver, kidney, spleen and heart) were weighed and preserved in formol saline. The remaining three animals in each group were observed for a further 48 h to determine any signs of delayed toxicity after which they were also euthanized.

Sub-acute toxicity studies

Twenty-four healthy rats (150 - 250 g) were divided into four groups ($n = 6$). One group served as control and received oral doses of 2% tragacanth. The other groups received daily doses of plant extracts (500, 1000 and 2000 mg/kg p.o) for 14 days. Animals in the various groups were observed over the study period, for changes in autonomic effects, behavioral responses, mortality and other signs of toxicity.

Autopsy was carried out on the animals that died during the study period. Surviving animals were euthanized at the end of the study to determine possible gross and microscopic lesions of vital organs. Blood samples obtained were collected from animals in the control as well as the treated groups on the last day.

Haematological analysis

Samples of blood were collected from the jugular vein into tubes containing 0.1 ml 4% aqueous solution of di-potassium salt of ethylenediaminetetraacetic acid (EDTA).

Erythrocyte count, total and differential leucocytes count, haemoglobin concentrations, haematocrit and other blood parameters were performed for both treated and control animals. The parameters were obtained with an auto analyzer (Cell-Dyne model. 331430 (Abbott Laboratories, IL, USA).

Biochemical assays

Blood samples were collected into tubes without an anticoagulant. The samples were centrifuged at 80 rpm for 10 minutes, and the serum was extracted into vials. This was used for the determination of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (AP), Gamma Glutamyl Transferease (GGT), Bilirubin (direct and indirect), total protein, albumin and globulin, and cholesterol in the sample. Urea and creatinine assays were also carried out to assess possible renal toxicity. The auto-analyzer, Random Access Chemistry System (Elan Diagnostics, Smithfield, RI, USA) was used for the analysis.

Determination of weights of animal and organs

Treated and control animals were weighed at 7 days intervals. At the end of the experiment, three rats from each group were euthanized and the liver, spleen, kidney, heart, stomach and intestines were harvested and weighed. The differences between means of absolute organ weights and the organ to body weight ratios were analyzed statistically.

Histopathological analysis

After euthanasia, major organs including the liver, kidney, spleen, heart, stomach and intestines were examined for possible pathological lesions. After macroscopic examination, representative fragments were subsequently fixed in a 10% buffered formalin solution (pH 7.4) and enclosed in liquid paraffin. Sections (5 μ M) were obtained and stained with haematoxylin-eosin for microscopic examination.

Statistical analysis

Statistical evaluation was performed using GraphPad Prism Ver.

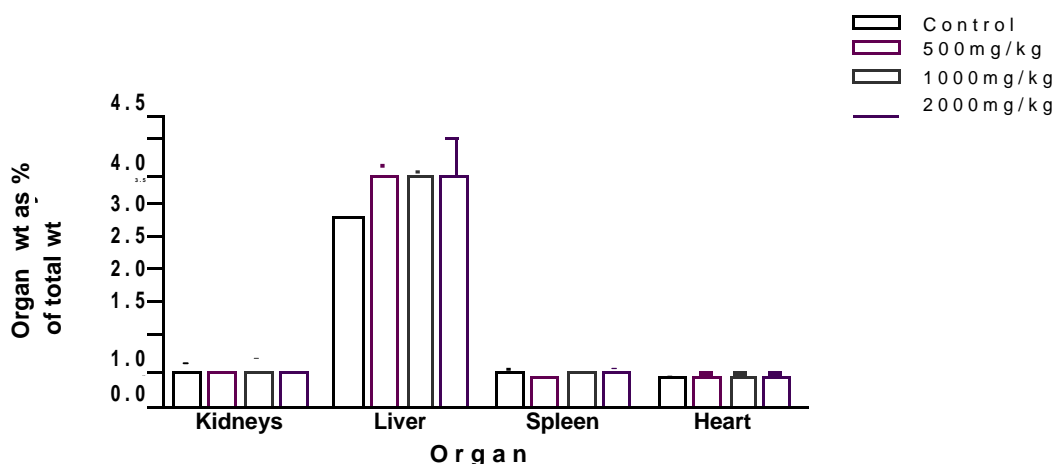


Figure 1. Relative organ weights of rats treated with *C. sanguinolenta* extract daily for 14 days. Following the treatment period, animals were euthanized and the organ/body weight ratios were determined. Relative weights compared to the control were not significant ($P > 0.05$).

5.0, for Windows XP. All results were presented as mean \pm S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) and, when appropriate, followed by Tukey's multiple comparison tests and p value < 0.05 was considered significant.

RESULTS

Effects of acute oral *C. sanguinolenta* extract

Throughout the period of the acute toxicity study, the treated rats showed no signs of toxicity. The rats were alert with no observable behavioural, motor or neurological changes. No gastrointestinal tract disorder, respiratory distress, locomotor activity or mortality was observed in any of the animals in the experiment. There was no significant effect on the body weights or on the weights of the major organs of the animals studied at all dose levels after 24 and 72 h.

Behavioural effects of 14-day administration of *C. sanguinolenta* in rats

Groups of rats receiving 1000 and 2000 mg/kg body weight of *C. sanguinolenta* for 2 weeks exhibited central nervous system (CNS) effects. Three rats in the group receiving 2000 mg/kg showed signs of walking backwards, circling, staggering, ataxia and loss of grip strength on day 5 of administration of the extract. Food and water intake was also considerably reduced. Two rats in the group receiving 1000 mg/kg of *C. sanguinolenta* also exhibited similar signs on day 7.

Effect on body weight

Changes in body weight for groups of rats that received *C. sanguinolenta* (500 - 2000 mg/kg) for 14 days were

not found to be significant in relation to the control groups.

Relative organ weight and macroscopic examination of organs

Two rats in the group receiving 2000 mg/kg died on day 6 and 7 respectively. While one of the rats in the group receiving 1000 mg/kg exhibited CNS toxicity and died on day 8. Autopsy examination of the dead rats revealed slight enlargement of liver and kidneys, with congestion and hyperemia in the lungs and muscles. The heart and the spleen however did not show any gross abnormality. No mortality occurred in the control or the remaining animals of the treatment groups. The physical appearance and behaviour of the surviving rats were comparable to the control group. Body/organ weight ratios (%) did not show significant differences between control and treated groups (Figure 1).

Effect of extract on haematological parameters

Rats receiving *C. sanguinolenta* (500 - 2000 mg/kg) orally over the period of 14 days did not show any significant changes in most of the haematological parameters assessed compared to the control. However, *C. sanguinolenta* increased platelet count significantly ($P < 0.05$) at all dose levels, with a profound increase ($P < 0.01$) in animals that received 1000 and 2000 mg/kg compared to the controls (Table 1). Additionally, granulocyte levels increased in a dose-dependent manner which was significant at the highest dose of 2000 mg/kg.

Effect of *C. sanguinolenta* on liver and kidney function

The 14-day subacute oral administration of *C. sanguino-*

Table 1. Haematological parameters of rats treated with *C. sanguinolenta* daily for 14 days.

Parameters	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
RBC (M/ μ l)	6.04 \pm 0.14	5.30 \pm 0.82	5.53 \pm 0.07	4.07 \pm 0.34
HB (g/dl)	9.77 \pm 1.22	13.90 \pm 0.29	12.42 \pm 2.57	12.80 \pm 0.00
HCT (%)	27.91 \pm 3.01	40.61 \pm 6.47	35.00 \pm 6.61	34.50 \pm 2.30
MCV (fl)	68.50 \pm 3.31	58.40 \pm 0.78	59.30 \pm 5.08	58.11 \pm 0.80
MCH (pg)	23.91 \pm 1.54	22.92 \pm 0.33	21.30 \pm 1.05	23.20 \pm 0.25
MCHC (g/dl)	34.90 \pm 0.61	39.33 \pm 0.21	34.70 \pm 5.55	39.92 \pm 0.10
PTL (K/ μ l)	353.00 \pm 49.40	770.00 \pm 13*	1000 \pm 0.00**	958.00 \pm 42.50**
WBC (K/ μ l)	14.43 \pm 5.07	16.87 \pm 5.07	16.87 \pm 5.07	8.46 \pm 1.23
LYM (%)	8.60 \pm 0.67	6.23 \pm 0.89	12.81 \pm 3.24	8.45 \pm 0.55
MID (%)	1.43 \pm 0.19	2.87 \pm 1.15	3.70 \pm 1.53	2.14 \pm 0.08
GRAN (%)	0.77 \pm 0.15	1.20 \pm 0.65	2.60 \pm 0.65	3.70 \pm 0.20**

Values are mean \pm S.E.M, n = 3, * Significant difference from the control (P 0.05), ** (P 0.01).

Table 2. Serum biochemistry of rats treated with *C. sanguinolenta* daily for 14 days.

Parameters	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
AST (U/l)	21.67 \pm 6.49	17.67 \pm 0.67	14.00 \pm 1.52	13.00 \pm 4.00
ALT(U/l)	80.67 \pm 13.17	83.30 \pm 8.41	102.7 \pm 11.67	95.50 \pm 10.50
AP(U/l)	241.70 \pm 42.33	179.30 \pm 2.60	280.7 \pm 79.83	363.50 \pm 15.50
GGT (U/l)	7.33 \pm 0.88	5.00 \pm 1.53	6.33 \pm 2.02	14.50 \pm 2.50
BIL Total (mg/dl)	0.90 \pm 0.21	1.63 \pm 0.71	1.43 \pm 0.46	1.35 \pm 0.15
BIL Direct (mg/dl)	0.17 \pm 0.03	0.63 \pm 0.26	0.80 \pm 0.70	0.25 \pm 0.05
BIL Indirect (mg/dl)	0.73 \pm 0.20	1.20 \pm 0.45	1.17 \pm 0.29	1.10 \pm 0.10
PROTEIN (g/dl)	8.30 \pm 0.45	8.56 \pm 0.17	7.36 \pm 0.37	8.25 \pm 0.35
ALBUMIN (g/dl)	3.63 \pm 0.14	3.70 \pm 0.15	3.767 \pm 0.29	3.25 \pm 0.05
GLOBULIN (g/dl)	4.66 \pm 0.34	4.83 \pm 0.17	3.77 \pm 0.29	5.00 \pm 0.30

Values are mean \pm S.E.M, n = 3, P was not significant at 95% level.

Table 3. Serum BUN and creatinine in rats treated with *C. sanguinolenta* daily for 14 days.

Parameters	Control	500 mg/kg	1000 mg/kg	2000 mg/kg
BUN (mg/dl)	30.10 \pm 4.25	28.53 \pm 0.88	24.77 \pm 2.83	24.70 \pm 2.90
CREATININE (mg/dl)	0.78 \pm 0.10	0.73 \pm 0.06	0.66 \pm 0.02	0.70 \pm 0.03
BUN / CREAT	40.33 \pm 4.67	39.67 \pm 2.72	38.0 \pm 4.58	35.5 \pm 2.50

Values are mean \pm S.E.M, n = 3, P was not significant at 95% level.

lenta (500 - 2000 mg/kg) induced no significant effects in serum enzymes, bilirubin, total protein, albumin and globulin levels (Table 2). Furthermore, subacute oral administration of *C. sanguinolenta* extract (500 - 2000 mg/kg) had no effect on the blood urea nitrogen (BUN) and creatinine levels (Table 3).

Histopathological findings

No alterations were observed in the organs of control and treated animals. No changes were found in the liver, kidneys, heart, spleen and gastro intestinal tract. There was also no observable necrosis, fatty degeneration or fluid accumulation in cells.

DISCUSSION

The potential of *C. sanguinolenta* in the management of animal diseases on the basis of its successful use in human diseases prompted us to undertake toxicity studies to assess its possible toxicity in rats.

The acute toxicity studies did not yield any adverse effects. All animals survived until the scheduled euthanasia, and no gross pathological lesions were detected in the internal organs. Organ weight assessment showed that at the doses used *C. sanguinolenta* did not produce organ swelling, atrophy or hypertrophy. The LD50 value for the freeze-dried extract of *C. sanguinolenta* from the present study was found to be higher than the 3000 mg/kg, suggesting a wide range of safety of the extract.

During the 14-day subacute test with *C. sanguinolenta* extracts, food consumption and body weights were considerably reduced. The CNS effects exhibited by the group of animals administered with *C. sanguinolenta* suggest that the extract might have some adverse effects on the CNS. Indeed work in our laboratory showed profound prolongation of sleeping time in rats treated with *C. sanguinolenta* (Ansah et al., 2008). This calls for caution in the routine use of the extract for the management of animal diseases. For the haematological analyses, most parameters assessed were similar to those of the control group. However, a significant increase in number of platelets at high dose levels (1000 - 2000 mg/kg) was observed. Cryptolepine, the major alkaloidal component of the plant *C. sanguinolenta* is known to inhibit platelet aggregation (Oyekan et al., 1988). It is however not clear if the increase in platelet counts shown by the extract in the present study is related to the findings of Oyekan and co-workers. High levels of platelets in the blood would suggest possible thrombocytosis and blockage of blood vessels. Autopsy examination of the dead rats revealed slight enlargement of liver and kidneys, with congestion and hyperemia in the lungs and muscle, suggesting inflammation. Although this correlated well with an increase in granulocytes at the highest dose, it did not reflect in differences between the relative organ weights of treated and control animals. Additionally, monitoring of hepatic function by serum transaminase measurements did not indicate possible hepatic damage. Elevations in AST are usually associated with cell necrosis in many tissues. For example, pathology involving the skeletal or cardiac muscles and/or hepatic parenchyma induces leakage of large quantities of this enzyme into circulation (Keneko, 1980; Bush, 1991; Duncan et al., 1994). ALT on the other hand is present in the liver cells and it is the specific marker for assessing liver cell damage. It is particularly useful in measuring hepatic necrosis, especially in small animals (Cornelius, 1989; Bush, 1991). Since it is one of the specific assayable liver enzymes, its elevation is associated with liver damage. In this study however, no significant increases in AST, ALT or GGT were observed in any of the animals dosed with *C. sanguinolenta*. This finding was supported by the lack of changes in the liver weights, and the histopathology in both treated and control animals. The lobular architecture and portal-space containing arterioles, venules and bile ducts for groups of animals dosed with extracts *C. sanguinolenta* were preserved.

Bilirubin (total, unconjugated and conjugated) and serum proteins were also assessed as a measure of liver function. Bilirubin is a major breakdown product of haemoglobin. With liver damage, the total bilirubin rises due to increase in unconjugated or conjugated fractions. When the conjugated fraction is elevated, the cause is usually extra-hepatic (Knoll, 1988). In the present study no elevations in bilirubin levels was observed confirming the pre-preservation of liver function. The extracts of the plants *C.*

sanguinolenta did not cause significant changes in the levels of serum total proteins, albumin or globulin compared to the control animals. Since the liver is the main organ of synthesis and secretion of proteins in the body, hepatic damage is usually associated with low levels of these proteins especially albumin. The result suggests that the plant extract did not affect protein synthesis at the dose levels studied.

Renal function was evaluated by measuring serum urea and creatinine levels. It should be noted that blood urea nitrogen (BUN) and creatinine are compounds in the blood derived from proteins, which are eliminated by the kidney and most often used to assess renal function (Iyayi and Tewe, 1998). High blood levels of BUN, creatinine and other metabolites are due to impaired excretion provoked by renal disease. Satyanarayana et al. (2001) reported that renal damage was observed only when creatinine and BUN increased concomitantly. The crude extract of the plant did not cause any changes in the serum levels of these biomarkers compared to the control. Moreover, in the histopathological analysis, no alterations in kidney morphology were observed. In all cases, renal cortex and renal corpuscles were preserved. Indeed, extracts of this plant was found to contain polyuronides such as tannins that possess antioxidant properties and consequently protect the renal tissues (Satyanarayana et al., 2001; Yokozawa et al., 1991). The beneficial effects of tannins against nephrotoxicity are also well known (Satyanarayana et al., 2001).

There are reports of cell damage caused by compounds isolated from *C. sanguinolenta* at the molecular level *in vitro* (Ansah et al., 2002, 2005; Humenuik et al., 2003). These reports *in vitro* may not always reflect *in vivo* as observed in the present study since pharmacokinetic and other factors have profound influences on the effects of toxicants *in vivo*.

All together, the results of our study indicate that doses of the extract up to 500 mg/kg are relatively safe. However, high doses of the extract (> 500 mg/kg) have the propensity to induce CNS toxicity, thrombocytosis and inflammation in target organs. We propose the use of *C. sanguinolenta* for animal diseases with these considerations.

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