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ACUTE AND SUBACUTE TOXICITIES OF THE AQUEOUS EXTRACT OF THE LEAVES OF *CELOSIA TRIGYNA* (L.)

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ABSTRACT

Celosia trigyna (C. trigyna) is a plant which is widely used in Burkina Faso as traditional medicine and human food. The aim of this study is to screen the phytochemical component of the extract and evaluate the safe of *C. trigyna* by acute and subacute toxicity with Wistar rat. During four weeks daily administration, subacute toxicity was investigated throughout consumption index, relative organs weight, biochemical and hematological parameters using 100, 500 and 1000 mg/kg body weight (bw). Phytochemical analysis was revealed the presence of steroidal and triterpenes, alkaloids salts and saponosides. For acute toxicity, the single administration dose of 5000 mg/kg of body weight was shown that the extract is practically non-toxic. For subacute toxicity 100, 500 and 1000 mg/kg were shown significant ($p < 0,05$) decrease of transaminases level. The different doses of the extract were also shown a significant ($p < 0,05$) decrease of chloremia and creatininemia. There is no significant ($p > 0,05$) change in biochemical parameters. For hematological parameters, only monocytes ratio arises significantly ($p < 0,05$) with 1000 mg/kg bw of the extract. From these results we conclude that *C. trigyna* extract in therapeutic short-term utilization did not present risk of toxicity but for long-term utilization, with higher doses could expose deleterious effects.

KEYWORDS: *Celosia trigyna*, Phytochemical screening, Acute toxicity, Subacute toxicity, Rat.

INTRODUCTION

Celosia trigyna (*Amaranthaceae*) is an herbaceous plant found in Nigeria, South Africa, at the Democratic Republic of the Congo. It is one of the most widely used plants in Africa traditional medicine and in human and animal nutrition.

Amaranthaceae family plants were known to present several and various pharmacological activities among which antioxidant, antitumor, antibacterial, anti-inflammatory, hypolipidemic, diuretic, antihypertensive, hypoglycemic, and analgesic activities. Especially for *C. trigyna*, its leaves are most often eaten by animals and consumed by the local people in Nigeria as soups and sauces.[1] Regarding its uses in traditional medicine, diseases treated are very diverse and involve lesions, inflammations, traumas, parasitosis, infections and allergies.[2,3,4,5,6,7,8] It is reported the antiulcerogenic effects on ethanol-induced gastric ulcers in adult Wistar rats.[1] Cytotoxicity of *C. trigyna* ethanol extracts on HeLa cells was also reported and classified this plant as highly cytotoxic for cervical adenocarcinoma with reference to cisplatin.[9] Other literature data revealed that *C. trigyna* contains flavonoids, steroidal saponosides, triterpenes, sterols, betalaïns, tannins, proteins, and amino acids, celosianines, chromogenic substances, and reducing compounds.[10,11,12,13]

In Burkina Faso, the decoction of leaves and stems of *C. trigyna* is traditionally used against constipation, dystocia, anorexia, urethritis, and has hemostatic, anti-inflammatory, diuretic effects.[14]

However, there are no sufficient results on the scientific basis of these various properties mainly in toxicity study. The purpose of present studies is to assess acute and subacute toxicological risks, related to plant alimentary consumption and its medicinal utilization.

1 MATERIALS AND METHODS

1.1 Materials

The experiment was carried out with female Wistar rats, provided by Laboratory of Animal Physiology of University Joseph KI-ZERBO. Animals were bred under controlled environmental conditions including temperature (22°C), relative humidity (50%) and photoperiod (light-dark cycle 12 hours/12 hours). Water and rodents pellet diet were

available *ad libitum*. All the experiments were achieved according to the ethic committee guideline for animal well-being of University Joseph KI-ZERBO.

The *C.trigyna* leaves were harvested in Loumbila, located at 18 km from Ouagadougou (Burkina faso). The plant sample has been authenticated by the laboratory of Biology and plant Ecology where the voucher specimen has been deposited under number: 6962.

1.2 Methods

1.2.1 Preparation of aqueous extracts

Celosia trigyna leaves were dried in the laboratory under artificial ventilation at room temperature. The dried sample of the plant was pulverized using a mechanical grinder. The powder obtained was used for extraction. The aqueous maceration of *C. trigyna* leaves powder was carried out according to current extraction techniques used in Phytochemistry Laboratory of the Institute for Research in Health Sciences of Ouagadougou (Burkina Faso). A test sample (500 g) of *C. trigyna* leaves powder was placed in a 5000 mL stainless steel beaker containing 1500 mL of distilled water. After homogenization, mixture was macerated at laboratory room temperature (30°C) for 24 hours under mechanical agitation. Macerated aqueous extract was then filtered, and centrifuged at 2000 rpm for 10 min. The supernatant was either directly used for phytochemical screening or collected and freeze-dried. Obtained powder (lyophilizate) is named aqueous extract of *C. trigyna* (AECT). It was weighted for extraction yield determination and stored at 4°C for experiments.

1.2.2 Phytochemical screening

The Phytochemical screening consisted to search some important chemical groups: steroidal and triterpenes glycosides, flavonoids, anthracenosides, coumarins and its derivatives, cardenolides, polyphenols (tannins), alkaloids salts, anthocyanosides, saponosides, and reducing compounds.[15]

1.2.3 Acute toxicity

Administration quantities of lyophilizate were dissolved in 0,9% of NaCl. Administrations was orally done using a probe connected to a syringe, and their volume was adjusted to 1 mL per 100 grams of animal body weight (bw).

Acute toxicity was conducted according to guideline 423 of OECD.[16] Six (06) female nulliparous 8-week-old rats were used, divided into 2 lots of 3 rats. Twenty-four (24) hours

prior AECT administration, the rats were fasted and 4 hours before, they were deprived of drinking water. The control group received NaCl (0.9%) and the second group received AECT solution at 5000 mg per kilogram of body weight (5000 mg/kg bw). Animals were first observed for one hour after AECT administration before being re-supplied with food and drinking water. Further observations were done after 1 hour, 24 hours, 48 hours, 72 hours, and 14 days. Mortality, drowsiness, lethargy, mobility, food and water intake, bristles ruffling, muzzle color, eye color, defecation types (diarrhea or not), convulsions, salivation, etc. were noted.

1.2.4 Subacute toxicity

Subacute toxicity was conducted according to guideline 407 of OECD.[17] Seventy-two (72) non-pregnant nulliparous females of 08 weeks old were used. The rats were divided into 6 groups of 6 rats, subjected to daily treatment (solution administrations) for 28 consecutive days:

- Control: administration of 1 mL/100 g of NaCl at 0.9% (NaCl).
- Lot 1: administration of AECT at 100 mg/kg bw (AECT 100);
- Lot 2: administration of AECT at 500 mg/kg bw (AECT 500);
- Lot 3: administration of AECT at 1000 mg/kg bw (AECT 1000);
- Satellite control: administration of NaCl;
- Satellite: administration of AECT 1000.

For satellite groups, after 28 days of treatment, it is added two weeks observation without any administration. They were useful for monitoring a possible reversibility, persistence, or late onset of toxic effects.

The rats were weekly weighed during the experimental period; water intake and food consumption were also measured, and consumption index was calculated as:

$$Consumption\ index = \frac{Food\ consumption}{Weight\ increase}$$

On the 29th day (two weeks later for satellite groups), the animals were anaesthetized with ketamine/xylazine (1.0/0.7) and blood was drawn by cardiac punctures from dry tubes and EDTA tubes. Blood of the dry tubes was centrifuged for 10 minutes at 3500 rpm to obtain plasma, used for biochemical assays, performed at Institute for Research in Health Sciences

using a spectrophotometer (mindray BA-88A). Measured parameters are creatinine, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), total protein, phosphorus, chloride, and calcium. Blood in EDTA tubes was used for figurative elements of blood study (hematological parameters) carried out at biomedical laboratory of Charles De Gaulle University Hospital Center of Ouagadougou.

After blood collection, rats were autopsied, and lungs, kidneys, spleen, liver, and hearts were taken and weighed and related to the whole animal's weight (relative organ weight).

1.3 Statistical analyses

The Excel 2016 spreadsheet was used to enter the data and calculate averages and standard errors of the mean. The graphs were created using Graph Pad Prism version 5.03 software. The one-factor analysis of variance (ANOVA I) followed by the Tukey-Kramer post-test using Graph pad was used to compare the data. The difference was considered as statistically significative, very significative, and highly significative when, respectively $p < 0.05$, $p < 0.0l$, and $p < 0.00l$.

2 RESULTS AND DISCUSSION

2.1 Phytochemical study

The phytochemical screening tests carried out in tubes revealed the presence of saponosides and alkaloids salts in total macerate aqueous extract. After hydrolyzed of AECT to isolate organic fraction, characterization revealed the presence of steroidal and triterpenes glycosides (Table 1).

Table 1: Phytochemical compounds of *Celosia trigyna.*

Total aqueous extract	
Saponosides	Presence
Alkaloids salts	Presence
Polyphenols (tannins)	Traces
Anthocyanosides	Not detected
Reducing compounds	Not detected
Organic fraction of aqueous extract	
Steroidal and triterpenes glycosides	Presence
Anthracenosides	Traces
Coumarins and its derivatives	Traces
Flavonoids	Not detected
Cardenolides	Not detected

Phytochemical results confirm literature data.[10, 11, 12, 13] Saponosides are heterosides and their main properties, among others, are antiproliferative, anti-inflammatory, and hepatoprotective.[18, 19, 20, 21] They are also characterized by their surfactant, cytotoxic, and hemolytic properties, generally attributed to their interaction with erythrocytic membrane sterols.[22] Alkaloids form an important group of natural substances of therapeutic interest through their structural diversity and the range of their pharmacological activities. Alkaloids have diuretic effect *via* their action on the renal epithelium and used as therapeutic against oedema.[23, 24]

2.2 Acute toxicity

Oral administration of the aqueous extract leaves of *C. trigyna* (AECT) at dose of 5000 mg/kg bw to rats did not cause any mortality. The observed effects during the 14 consecutive days did not differ from those of the first hours of first day. We conclude that LD_{50} is greater than 5000 mg/kg bw, that classify *C. trigyna* as practically non-toxic plant.[25] This conclusion is in accordance with the Globally Harmonized System of Classification and Labelling of Chemicals of OECD,[17] where AECT can be classified in category 5 (non-toxic substance).

2.3 Subacute toxicity

Subacute toxicity results relate to consumption index, relative weight change of lungs, heart, liver, kidneys and spleen, biochemical and hematological parameters.

2.3.1 Effects of aqueous extract of *Celosia trigyna* administration on consumption index

For all used doses, results did not show significative variation of consumption index, compared to control. This result is maintained after two weeks additional observations without AECT administration (Fig. 1).

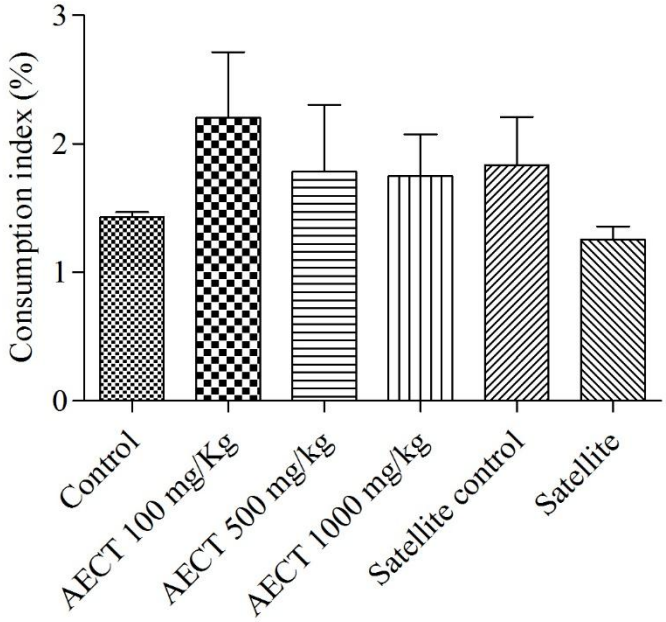

Figure 1: Rat consumption index at 28th day after daily aqueous extract of *Celosia trigyna* administration (n = 6).

Index consumption is zootechnical parameter that indicate alimentation efficiency in animal's weight increase. In our experiments, AECT administration did not affect rat ponderal growth.

2.3.2 Relative organs weight

For all organs and at all used doses, there was no significative variation of relative organs weight (Table 2).

Table 2: Effect of aqueous extract of *Celosia trigyna* on relative weights of rat organs.

	Lungs	Heart	Liver	Kidneys	Spleen
Control	0.67 ± 0.05	0.38 ± 0.01	3.52 ± 0.26	0.69 ± 0.03	0.27 ± 0.01
AECT 100 mg/kg bw	0.53 ± 0.01	0.30 ± 0.01	3.20 ± 0.24	0.62 ± 0.03	0.21 ± 0.01
AECT 500 mg/kg bw	0.60 ± 0.04	0.31 ± 0.00	3.10 ± 0.10	0.61 ± 0.01	0.25 ± 0.01
AECT 1000 mg/kg bw	0.63 ± 0.02	0.37 ± 001	3.22 ± 0.15	0.68 ± 0.03	0.27 ± 0.02
Satellite control	0.50 ± 0.03	0.28 ± 0.01	3.38 ± 0.15	0.56 ± 0.02	0.22 ± 0.01
Satellite	0.50 ± 0.02	0.30 ± 0.01	3.65 ± 0.15	0.61 ± 0.01	0.24 ± 0.00

Values are expressed as mean ± standard error to the mean (SEM); n = 6.

AECT administration did not affect relative organ's weight in our experiments. Similar results are found about aqueous extract of *Excoecaria grahamii* or *Senna alata*.[26,27]

2.3.3 Biochemical parameters

Plant extract utilization can affect the physiology of many organs such as liver and kidneys. For completion of subacute toxicity study, it is important to conduct a biochemical assessment.[28, 29, 30, 31] Our experiments targeted blood levels of transaminases, chloride, phosphorus, creatinine, and calcium. The main changes we observed were those of transaminases. AECT induced a highly significative ($p < 0.001$) decrease of ASAT blood levels. This decrease was maintained in satellite group, after additional two (2) weeks observation without AECT administration (Fig. 2).

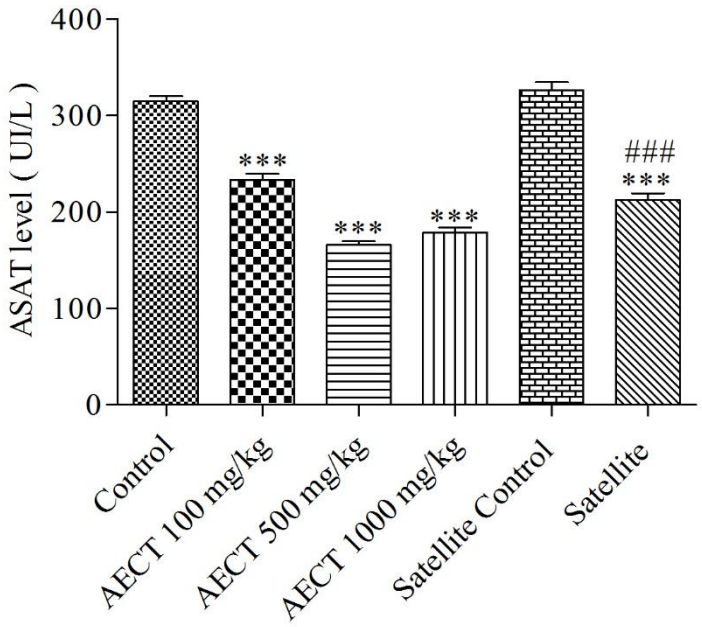

Figure 2: Aqueous extract of *Celosia trigyna* effect on ASAT levels (n = 6). *Compared to control: p < 0.05 (*), significative; p < 0.0l (**), very significative; p < 0.00l (***), highly significative. Compared to satellite control: p < 0.05 (#), significative; p < 0.0l (##), very significative; p < 0.00l (###), highly significative.*

The same result was obtained for ALAT level (Fig. 3). There was a high significative decrease of ALAT level when AECT administered. This decrease was very significative in satellite batch.

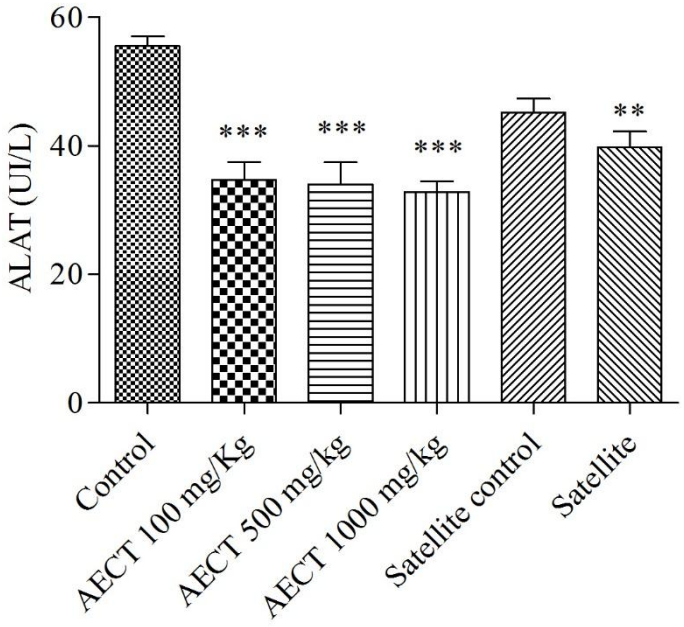

Figure 3: Aqueous extract of *Celosia trigyna* effect on ALAT levels (n = 6). *p < 0.05 (*), significative; p < 0.0l (**), very significative; p < 0.00l (***), highly significative compared to control.*

Our results show a highly significative decrease of ALAT and ASAT levels induced by AECT administration. Similar results were obtained with aqueous extracts of the leaves respectively of *Eleophorbia drupifera* and Artemisia afra in rats.[32,33] This result would express hepatoprotective properties of AECT. ALAT, a more specific indicator of hepatoprotective effect.[34, 35] is located primarily in the cytosol of hepatocytes however, and ASAT is found in cytoplasm and mitochondria of various other organs tissues, and in erythrocytes.[36] This probable hepatoprotective effect of AECT may be due to saponosides properties as suggested by some authors.[20, 21]

For chloride level, we observed a very significative decrease ($p < 0,001$) after AECT 500 and AECT 1000 administrations. This decrease is maintained in satellite and control satellite groups after supplemental 14 days without extract administration (Fig. 4).

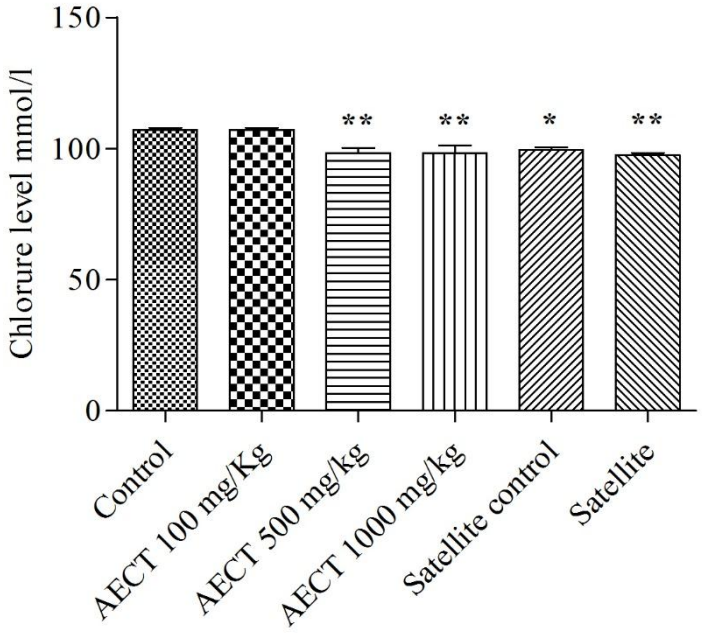

Figure 4: Aqueous extract of *Celosia trigyna* effect on chloride level (n = 6). *p < 0.05 (*), significative; p < 0.0l (**), very significative compared to control.*

The usual values of chlorine (in human) are between 90 and 100 mmol/L.[37] Its decrease could be explained by sodium intake deficiency, or diuretic property of plant extract.

We also found that phosphorus and creatinine levels decreased significantly ($p < 0.05$) at the higher dose (AECT 1000). In both cases, these decreases are reversible and disappeared in the two satellite groups (Table 3).

Table 3: Effect of aqueous extract of *Celosia trigyna* on some biochemical parameters after twenty-eight days of treatment.

	Calcium (mmol/l)	Phosphorus (mmol/l)	Total protein (g/dl)	Creatinine (µmol/l)
Control (NaCl)	2.46 ± 0.03	5.30 ± 0.66	69.66 ± 3.45	71.86 ± 2.62
AECT 100 mg/kg bw	3.35 ± 0.30**	3.99 ± 0.35	67.25 ± 1.41	61.41 ± 2.16
AECT 500 mg/kg bw	2.44 ± 0.15	3.87 ± 0.56	55.43± 1.81*	61.26 ± 2.52
AECT 1000 mg/kg bw	2.21 ± 0.05	3.09 ± 0.29*	57.31 ± 2.20	59.05 ± 2.53*
Satellite control	2.52 ± 0.11	3.81 ± 0.30	65.61 ± 2.42	65.63 ± 4.02
Satellite	2.62 ± 0.11	4.10 ± 0.22	70.08 ± 5.27	63.25 ± 2.85

Values are expressed as mean ± standard error to the mean (SEM); n = 6.

Generally, decrease of phosphoremia is related to many causes; for example, redistribution of phosphorus to the intracellular compartment induced by catecholamines, or its depletion by

renal excretion induced by diuretics and glucocorticoids.[38, 39] Catecholamines, precursor of isochinolic alkaloids,[40] and diuretics may be present in AECT.

Creatinine, it is a physiologically inert nitrogen molecule, a waste product from muscle metabolism and tissue breakdown, issued from creatine. Usual normal values according to different author or laboratories are between 45 and 106 µmol/L.[41, 42] Creatinine is the best endogenous marker of glomerular filtration.[43] Its increase or decrease may reflect, respectively, renal failure or muscle atrophy.[44, 45, 46] Our result suggests a reversible muscle atrophy when higher dose of AECT is administered.

2.3.4 Hematological parameters

All data are normal compared to control except a significant increase of monocytes ratio at AECT 1000 mg/kg bw. This increase disappeared for satellites groups (Table 4).

Table 4: Effect of aqueous extract of *Celosia trigyna* administration on some hematological parameters.

	Hb (g/dl)	WBC (10^3/µL)	Plt (10^3/µL)	Lym (%)	Mono (%)	Gra (%)
Control	13.86 ± 0.30	2.25 ± 0.29	720.83 ± 27.03	83.26 ± 2.48	7.86 ± 0.52	7.86 ± 1.29
AECT 100 mg/kg bw	13.63 ± 0.28	2.83 ± 0.58	726.16 ± 16.58	87.66 ± 1.14	6.03 ± 0.49	6.30 ± 0.82
AECT 500 mg/kg bw	13.45 ± 0.25	2.41 ± 0.41	748.16 ± 21.53	84.90 ± 1.45	7.96 ± 0.41	7.13 ± 1.51
AECT 1000 mg/kg bw	14,08 ± 0.32	1.80 ± 0.07	775.66 ± 18.96	78.28 ± 4.23	10.60 ± 0.33*	8.53 ± 1.79
Satellite control	13.61 ± 0.31	3.33 ± 0.50	678.50 ± 19.66	81.91 ± 2.36	7.01 ± 0.82	11.06 ± 1.5
Satellite	13.48 ± 0.18	3.45 ± 0.32	690.66 ± 29.34	88.58 ± 1.32	5.31 ± 0.55	6.10 ± 0.83

WBC: White blood cells, Hb: Hemoglobin, Lym: Lymphocytes, Gra: Granulocytes, Mon: Monocytes, Plt: Platelets. Values are expressed as mean ± standard error to the mean (SEM); n = 6.

Monocyte normal ratio, in adult human is generally comprised between 2 and 10%. Our result could be considered as monocytosis which could be due to transitory inflammation or infection related to experimental conditions, excluding AECT intrinsic effect.

3 CONCLUSION

Our results corroborated the innocuity of plant consumption and its utilizations in traditional medicine. There was no acute toxicity and no significant effect on consumption index and some noble organs weight, and most biochemical and hematological parameters did not

significatively change. On the contrary, AECT has hepatoprotective effect (transaminases decrease) are found and diuretic properties is suspected (hypochloremia).

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