TOXICITY TESTING OF ENDOD, A NATURAL PLANT EXTRACT, AS A PREREQUISITE FOR ITS SAFE USE AS A MOLLUSCICIDE

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INTRODUCTION

Schistosomiasis (bilharzia) is a widely spread parasitic disease in Zambia and in other parts of Africa, the Middle and Far East, and South America. It infects approximately 300 million persons (WHO, 1980; Doumenge et al., 1987). As water contact is the means of transmission especially women and children are at risk and mostly infected. Schistosomiasis disease leads to kidney, urinary tract, liver manifestations giving rise to malnutrition, liver and kidney diseases, and cancer. Traditionally, the incidence of this disease has been low along waterways where laundry has been washed with berries of the Endod (*Phytolacca dodecandra*) plant. This has led to scientific investigations on the use of the extract of Endod berries as a molluscicide to prevent schistosomiasis transmission via the snail host (Lemma, 1990). Later Lemma et al., (1991) reported that Endod extract is an effective molluscicide also against zebra mussels at very low concentrations. However, the obstacle to its wider use has been the lack of sufficient toxicological data on its safety.

Although the efficacy of the Endod powder is unquestionable as a molluscicide (Lugt, 1986), its toxicological safety to the environment and human beings has not been proven. Passing toxicological tests will facilitate the implementation of Endod in bilharzia prevention. Recently, the acute toxicity of extract from the Ethiopian variety of Endod was tested both in aquatic species and in rodents (Lambert et al.,1991). The acute toxicity to aquatic species was rather high while the rodent toxicity was low.

We have conducted a subchronic (90-day) toxicity study according to OECD guidelines in rats using a Zambian variety of *Phytolacca dodecandra*. The main results of this study are reported here.

HPLC Analysis

The HPLC run of the Endod extract was made with LKB 2150 pumps, LKB 2152LC controller, LKB 2220 recording integrator, LKB 2151 variable wavelength integrator and Rheodyne 7125 injector with a 20 _l loop and a Spherisorb S5 C8 column. The operational circumstances were as follows. In the isocratic run as a running solution acetonitrile: 0.05 M ammonium dihydrogen-phosphate buffer, pH 4.4 was used at a ratio of 40:60. One pump provided acetonitrile-buffer solution (ratio 50:50) and another plain buffer. The flow rate was 1.5 ml/min, and the running time was 15 min. The detection wavelength was 203 nm. The Endod powder sample was eluted through a C18 (Varian) column, which was activated by 10 ml of methanol and washed with 10 ml of distilled water. The sample (5 mg of Endod extract in 10 ml of distilled water) was run slowly into

the column and then washed with 10 ml of water followed by a wash with 10 ml of methanol:water(1:4) solution. The elution was made with 5 ml of methanol, which was then evaporated under nitrogen flow. The dried sample was diluted in 5 ml of isopropanol:water (1:9) solution. Finally, the sample was filtered through a 0.22 _m filter (Gelman Sciences). In the chromatogram three major peaks were found with the retention times of 4.28, 5.81 and 8.32 min. The repetition of assays from different batches of sample preparation was good suggesting that the reproducibility of the spray-dryer method was sufficient. Based on the areal relationships at constant absorption coefficients, about 15 percent of the preparation is composed of these three active compounds.

MATERIALS AND METHODS

Production of Endod Powder

A standard procedure for the preparation of a water extract of Endod was finalised. As starting material predried *Phytolacca dodecandra* berries grown at the Kitwe Forest Station of the National Council for Scientific Research were used.

The Endod extract was purified by modifying a spray-dryer method. The method is sufficient for the laboratory scale production of powder. Powder of Endod extract was prepared for the experiment from the berries shipped from Zambia and grown at Kitwe Forest Station of NCSR. The berries were predried at 37_C stable weight. Thereafter the berries were homogenized into the particle size of 0.2 mm. The homogenate was soaked in distilled water (200 g homogenate/2 l water) for 24 hr by mixing it occasionally. The suspension was filtered through a cloth filter and a Buchner filter. The filtered suspension was transferred into the Buchi mini spray dryer which was then operated at pretested optimal conditions. The particle size distribution is given in Figure 1.

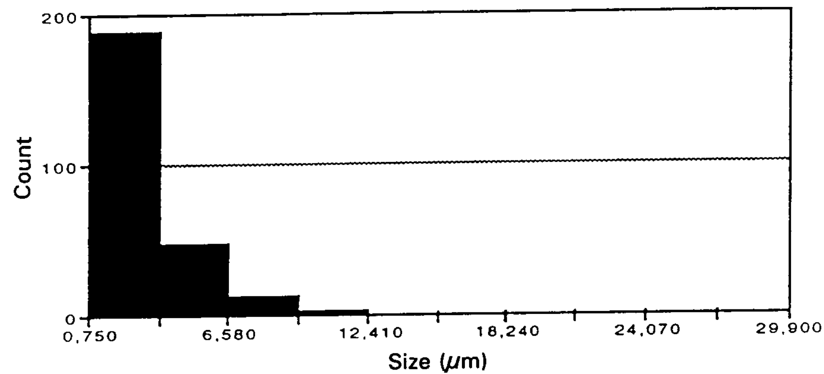

Figure 1. The particle size distribution of the Endod powder prepared

Animals

Dosing

Both male and female Wistar rats (15 rats/group) weighing 97-171 g were used at the age of 6 weeks. The rats were kept in cages of 4-5 rats at 20_C, and the humidity of the animal rooms was 60 percent. It was recorded regularly. The rats had free access to drinking water and food. Commercial pelleted food was given. Endod solutions were made at the following concentrations: 0, 5, 25 and 125 mg/ml. The solutions were approximately for one week at a time and kept at 4_C. Aqueous solutions of the Endod powder were given to rats by a gavage at the doses of 0, 50, 250 and 625 mg/kg of body wt 5 days /week for 13 weeks. At first the highest dosage group was given 1250 mg/kg but as these rats were dying, the dose was lowered in a few days.

In the experiments on acute toxicity, 4 month old rats and mice of both sexes were taken, 5 animals/group. The animals were given Endod aqueous solutions in concentrations of 50 mg/ml and 250 mg/ml by gastric gavage at doses of 0, 1000 and 2500 mg/kg as a single dose and the symptoms and survival of animals were followed.

Inspection of animals

Each working day, all rats were inspected and all observations were recorded. All of the rats were weighed weekly.

Blood samples and clinical chemistry

Blood samples were taken by venous punctures into capillaries at 10, 28 and 90 days for haematological and clinical chemistry analyses. At 28 days 3-5 rats/group were sacrificed for the pathological analyses and the rest of the animals were sacrificed at 90 days. Before sacrificing the specimens, blood was taken by a heart puncture.

Clinical analyses included electrolytes, liver function tests, creatinine, albumin and glucose. Haematology, including white blood cell counts, was also analyzed. All enzyme analyses were performed according to established clinical chemistry standards
OE, 1974), and the laboratory participated in a quality control program. The enzyme and other blood chemistry assays were performed according to the application notes of Kone Company (1985).

Necropsy samples

After 28 days, 3 rats in each group were sacrificed and macroscopic examination of all organs was performed. Major organs were placed in a 10 percent formalin to prepare histological slides. The other animals (12 rats per group) were sacrificed after 90 days in the experiment. Then all tissues were inspected and taken into formalin for histology. The slides were stained by haematoxoylin-eosin.

Liver tissue

A small piece of liver was taken for metabolic (enzyme induction of cytochrome P450 catalyzed reactions) studies. Microsomes were prepared by homogenizing the liver pieces in 4x wet weight of 0.15 M KCl-10mM K_2EDTA buffer, pH 7.4 (adjusted with KOH) with a Potter-Elvehjem type glass-Teflon homogenizer. After a 12000 g centrifugation for 15 min, the supernatant was taken for further centrifugation at 105000 g x 60 min and the pellet was resuspended in 200 mM KH_2PO_4-1 mM K_2EDTA-1mM DTT (dithiothreitol) buffer, pH 7.4 to wash the microsomes by recentrifugation at 105000 g x 60 min. Then the pellet was resuspended in the original wet weight in 200 mM K-phosphate-20 percent glycerol-1mM DTT buffer, pH 7.4 and the microsomes were stored at -80_C.

Cytochrome P450 concentrations in the microsomes were measured with a Shimadzu double beam spectrophotometer by scanning the carbon monoxide difference spectrum at 400-500 nm in the presence of sodium dithionite as described by Omura and Sato (1964). The microsomal ethoxyresorufin O-deethylase activity was measured in the livers of rats given Endod for 28 days. The activity was measured flurometrically with ethoxyresorufin and microsomes in the cuvette and the reaction initiated with 10 _l of 10 mM nucleotide adenosine diphosphate (NADPH) in the total volume of 1 ml. The deethylation of ethoxyresorufin was monitored by using an excitation wavelength of 530 nm and emitting a wavelength of 585 nm as described earlier by Prough et al.(1978). The microsomal protein contents was measured as described by Gornall et al. (1949).

Statistics

In all results the mean Å standard deviation (SD) is given if not specified otherwise, the student´s t-test was used to calculate statistical significances of the differences.

RESULTS

Physico-chemical Properties of the Endod Powder

Physico-chemical properties, including the melting point and water-oil partition have been determined. The melting point was determined with Meltemp II equipment. As the compound is composed of many different molecules, the melting point is not exact. Melting started at 200_C (already at 195_C some decomposition took place), and at 270_C melting and blackening were complete. The compound darkened while melting.

Solubility experiments of the Endod extract were conducted at 20_C and the Endod distribution in different phases was determined spectrophotometrically (LKB Ultrospect II) at a wavelength of 203 nm . The solubility in cyclohexane was 1.4 x $^{-3}$ g/l, xylene 5.7 x 10^{-3} g/l and water 32.58 g/l.

In an n-octanol/water distribution experiment at room temperature the organic and water phases were allowed to separate and samples were taken from both phases. The solvent was evaporated and the dry material was dissolved in water. Then the Endod

concentration was etermined spectrophotometrically. As a result 60 percent of the Endod was dissolved in water and 40 percent in n-octanol at the concentrations tested (0.01, 0.1 and 1.0 g/l).

Ultraviolet spectra were obtained for Endod concentrations ranging from 0.01 g/l and 1 g/l with a Shimadzu UV-2100 spectrophotometer. This gave a peak absorbance at 203 nm and a notch at 260 nm.

Toxicological Studies

Acute toxicity in rats and mice

The acute toxicity to rats and mice was assessed by giving 0, 1000 and 2500 mg/kg of body wt of Endod in distilled water at 50 mg/ml and 250 mg/ml concentrations by gavage, afterwhich the animals were monitored for 3 days. The LD_{50} values were determined in rats and mice of both sexes. The animals were 4 months of age and each group was composed of 5 animals. In rats the LD_{50} was calculated to be 1000 mg/kg for males and 920 mg/kg for females. In mice the LD_{50} was 1600 mg/kg for males and 3280 mg/kg for females. Toxic symptoms evoked in rats and mice were excessive salivation, drowsiness, and coarse hair.

Subacute and subchronic toxicity

Weight gain

After 28 days of Endod administration to male and female rats, no dose-related differences in body weight gain were found. Male rats given Endod at 250 mg/kg or 625 mg/kg doses had lower body weights than controls after 90 days while in females only at the highest dose was a decreased body weight apparent. Organ weights demonstrated no dose-related changes.

Haematology

No differences in any of the haematological parameters were found after 10 day dosages of Endod, either to males or females at any of the doses. After 28-day dosages of Endod into male rats there was a small but significant decrease in the 50 mg/kg group in total white blood cell and lymphocyte counts, but decreases were not evident at the higher doses. At this dose level there was also a transient decrease in blood haemoglobin concentrations in males. Also, in females small, transient changes in some haematological parameters were found which did not show any dose-responsiveness. After 90 day exposures, there was a transient decrease in blood thrombocytes in male rats given Endod at the dose of 50
mg/kg and in both males and females there were changes in blood lymphocytes (Table 1). In males, the lymphocyte percentage of total white blood cells decreased and in females an increase in both lymphocyte and white blood cell counts were found at the two highest doses (Table 1).

Clinical chemistry

After 10 day dosages of Endod extract to rats there was an increase in alanine aminotransaminase (ALAT) and aspartine amino transaminase (ASAT) activites, ALAT level was increased at the two highest dose groups and ASAT was increased at the highest dose group in both males and females. However, in gamma-glutamyl transferase

activity no increase due to the Endod exposure was found after 10 days. Bilirubin concentration was increased at the highest dose group in males after 10 day dosages of Endod. In urea there were transient changes which were not consistent with doses.

After 28 days, both ASAT and ALAT increased at all doses in males; but in females only small changes in two highest dose groups were found (Table 2). Blood glucose concentrations decreased in males at the highest Endod dose, but in females there were only transient changes. In serum calcium concentrations small changes in males were found; in females a transient increase was present.

After 90 day exposures a small but significant increase in ASAT activity was found in the two highest dose groups (Table 3). In males small changes were found in serum sodium levels in the two highest dose groups, and in serum phosphorus small variations were present (Table 3). No uniform dose-responsiveness was present. In females, urea was transiently increased in the 50 mg/kg dose group; albumin was slightly decreased in the highest dose group, and chloride was increased and phosphorus decreased at all doses (Table 3).

Pathology

In gross anatomy a few stomach irritation cases in both male and female rats in exposed groups occurred, but showed no consistency with dose. Coarse hair was found in some animals at the highest dose group. Histological examinations of organs revealed stomach mucosal haemorrhages in the two highest dose groups in both sexes. Otherwise, no dose-dependent changes in any of the tissues were found.

Enzyme Activities

In a 28-day experiment no inducibility in the liver cytochrome P450 concentration was found (in males the concentrations were as follows: controls; 14.5 Å 0.5 nmol/g; dose 50 mg/kg: 11.0 Å 4.7 nmol/g; dose 250 mg/kg: 9.0 Å 0.9 nmol/g). The cytochrome P450 concentration was also measured in the liver microsomes of rats treated with Endod extract for 90 days at various doses, but no significant changes were shown. No inducibility was found for ethoxyresorufin O-deethylase activity of liver microsomes.

DISCUSSION

Previously, Tier 1 studies were conducted on the Ethiopian variety of *Phytolacca dodecandra* plant extract, prepared as an aqueous extract similar to our study (Lambert et al., 1991). In this study where Endod powder was given to rats up to the dose of 500 mg/kg by gavage for 28 days, no signs of toxicity were found. However, Endod-S was a severe eye irritant and a slight irritant to rabbit skin (Lambert et al.,1991). Endod-S was not considered to be mutagenic in an Ames test or in the sister chromatid exchange test.

Table 1. Haematological parameters in male and female rats given Endod extract for 90 days.

PARAMETER	GROUP D CCONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP 625 mg/kg
MALE RATS				
RBC x 10^{12}/l	8.6Å0.6	8.9Å0.7	8.4Å0.6	8.8Å1.3
Hb (g/l)	164.0Å7.2	169.1Å22.0	163.6Å5.3	165.0Å11.9
Haematocrit(%)	0.44Å0.02	0.45Å0.04	0.43Å0.02	0.44Å0.06
MCV(fl)	50.0Å2.5	50.7Å1.0	51.0Å1.7	50.6Å1.5
MCH(pg)	19.1Å0.9	19.0Å1.3	19.4Å0.9	19.1Å2.2
MCHC(g/l)	378.6Å7.7	374.2Å27.9	381.4Å0.1	376.7Å37.4
WBC x 10^{9}/l	8.3Å2.5	10.4Å2.7	9.5Å3.0	8.6Å2.9
Lymphocyte x 10^{9}/l	7.8Å2.3	8.6Å1.8	8.6Å2.4	7.9Å2.7
Lymphocyte (%)	94.7Å2.1	84.5Å6.9***	90.8Å5.2*	91.2Å2.7**
Thrombocyte x 10^{9}/l	614.2Å141.8	239.7Å160.8***	541.7Å251.9	672.7Å266.3

Mean Å SD are given. For statistical significances:*=p<0.05; **=p<0.01; ***= p<0.001

Table 1. Continued				
PARAMETER	GROUP D CONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP C 625 mg/kg
FEMALE RATS				
RBC x 10^{12}/l	7.7Å0.4	7.3Å0.8	7.6Å0.6	7.5Å1.0
Hb (g/l)	157.2Å6.3	148.5Å16.0	153.5Å10.7	151.3Å16.8
Haematocrit(%)	0.41Å0.02	0.38Å0.04	0.40Å0.03	0.40Å0.05
MCV(fl)	53.5Å1.6	52.9Å1.5	52.4Å1.4	53.1Å1.1
MCH(pg)	20.5Å0.9	20.5Å0.6	20.2Å0.7	20.2Å0.8
MCHC(g/l)	383.1Å11.9	383.3Å12.6	384.8Å9.1	379.8Å12.4
WBC x 10^{9}/l	5.1Å1.0	6.5Å3.2	7.3Å2.4**	9.1Å3.1**
Lymphocyte x 10^{9}/l	4.8Å1.0	5.8Å2.5	6.7Å2.2*	8.5Å2.9**
Lymphocyte %	93.9Å1.8	89.7Å4.2**	91.5Å1.6**	92.8Å3.4
Thrombocyte x 109/l	473.3Å154.0	369.8Å252.4	565.5Å228.6	589.8Å314.3

Mean Å SD are given. For statistical significances:*=p<0.05; **=p<0.01; ***= p<0.001

Table 2. Clinical chemistry in blood of rats given Endod extract for 28 days in male and female rats.

PARAMETER	GROUP D CONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP C 625 mg/kg
MALE RATS				
Alk.phosphatase(IU)	387Å127	292Å39	252Å43	N.D.
ALAT (IU)	60.3Å7.0	53.0Å13.9	68.0Å8.5	125.3Å43.9
ASAT (IU)	159.4Å25.6	208.8Å56.7*	204.6Å37.3**	194.0Å33.3*
G-GT (IU)	3.1Å2.9	9.2Å10.9	8.6Å16.7	3.2Å2.4
Bilirubin (mmol/l)	13.4Å4.6	14.2Å9.5	16.4Å6.6	11.8Å3.4
Creatinine (mmol/l)	56.2Å4.3	61.0Å5.2*	67.9Å10.8*	55.1Å4.9
Urea (mmol/l)	7.1Å0.7	6.9Å1.1	6.9Å0.8	5.4Å0.7***
Albumin (%)	13.7Å1.7	15.2Å2.1	16.8Å2.0**	14.7Å1.9
Glucose (mmol/l)	8.4Å0.4	9.3Å2.6	9.3Å1.4	5.4Å0.8***
Sodium (mmol/l)	157.7Å2.9	156.3Å2.1	164.7Å3.8	N.D.
Potassium (mmol/l)	6.3Å0.2	6.4Å0.5	6.2Å0.6	N.D.
Chloride (mmol/l)	109.7Å4.0	110.7Å4.2	124.7Å6.4*	N.D.
Calcium (mmol/l)	3.00Å0.07	2.87Å0.02	2.98Å0.03	N.D.
Phosphorus(mmol/l)	2.46Å0.34	2.69Å0.31	2.30Å0.26	N.D.

Table 2. d

PARAMETER	GROUP D CONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP C 625 mg/kg
FEMALE RATS				
Alk.phosphatase(iu)	202.3Å42.4	149.0Å32.2	141.3Å38.3	143.3Å28.7
ALAT (IU)	57.5Å6.7	62.3Å6.2	60.0Å7.6	72.0Å21.1*
ASAT (IU)	175.5Å28.2	205.3Å51.1	197.9Å24.0*	195.6Å22.5
G-GT (IU)	2.0Å2.6	9.9Å19.9	3.9Å3.2	1.3Å2.3
Bilirubin (mmol/l)	13.6Å3.9	17.8Å4.2*	17.2Å4.6	14.4Å4.1
Creatinine (mmol/l)	63.3Å4.3	66.1Å6.6	63.1Å6.0	63.6Å7.7
Urea (mmol/l)	8.3Å0.7	7.6Å0.9*	7.4Å1.2*	7.1Å1.3*
Albumin (%)	18.6Å2.2	19.0Å2.1	17.3Å2.0	17.0Å2.1
Glucose (mmol/l)	5.2Å0.8	7.0Å1.2***	4.7Å0.9	3.6Å0.7***
Sodium (mmol/l)	156.7Å3.5	160.3Å4.2	157.3Å2.3	155.7Å4.2
Potassium (mmol/l)	6.23Å0.51	6.73Å0.55	6.73Å0.57	6.83Å0.75
Chloride (mmol/l)	97.3Å2.1	115.7Å6.4**	121.0Å12.2*	109.7Å2.1**
Calcium (mmol/l)	3.01Å0.02	2.86Å0.10	2.88Å0.15	2.85Å0.12
Phosphorus (mmol/l)	2.60Å0.27	2.01Å0.27	2.51Å0.25	2.21Å0.43

Mean Å SD are given. For statistical significances: *=p<0.05; **=p<0.01; ***=p<0.001

Table 3. Clinical chemistry in blood of male and female rats given Endod extract for 90 Days.

PARAMETER	GROUP D CONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP C 625 mg/kg
		MALE RATS		
Alk.phosphatase(iu)	219.6Å78.9	207.2Å57.2	216.9Å73.6	217.4Å65.7
ALAT (IU)	40.5Å17.3	9.8Å18.2***	36.2Å24.3	19.0Å33.0
ASAT (IU)	96.8Å25.7	119.2Å29.5	131.3Å28.9*	137.2Å81.7
G-GT (IU)	0.0Å0.0	0.0Å0.0	0.1Å0.3	0.4Å0.5*
Bilirubin (mmol/l)	4.0Å1.1	4.1Å0.8	3.9Å0.7	5.4Å5.5
Creatinine (mmol/l)	57.5Å4.2	55.3Å6.5	53.9Å6.2	59.8Å3.1
Urea (mmol/l)	8.2Å0.9	7.5Å0.9	7.5Å1.2	6.1Å0.9**
Albumin (%)	14.9Å2.5	15.7Å3.3	16.9Å4.1	13.2Å1.9
Glucose (mmol/l)	7.5Å0.6	7.1Å0.6	7.5Å1.1	8.3Å2.4
Sodium (mmol/l)	147.4Å1.3	147.1Å0.7	146.2Å1.1*	149.4Å1.1**
Potassium (mmol/l)	6.3Å0.6	6.4Å0.6	6.2Å0.5	6.2Å0.4
Chloride (mmol/l)	85.5Å9.6	93.6Å9.8	96.0Å8.6*	95.2Å8.4
Calcium (mmol/l)	3.01Å0.24	3.39Å0.69	3.34Å0.61	2.78Å0.09
Phosphorus (mmol/l)	2.67Å0.30	3.27Å0.59**	2.39Å0.37**	2.45Å0.29

Mean Å SD are given. For statistical significances:*=p<0.05; **=p<0.01; ***= p<0.001

Table 3. Continued				
PARAMETER	GROUP D CONTROL	GROUP A 50 mg/kg	GROUP B 250 mg/kg	GROUP C 625 mg/kg
FEMALE RATS				
Alk.phosphatase(IU)	128.9Å42.0	124.2Å55.9	114.3Å35.0	127.6Å41.7
ALAT (IU)	7.8Å15.9	23.2Å21.1	54.0Å42.4**	11.1Å8.6
ASAT (IU)	106.1Å25.9	109.4Å53.8	129.8Å16.5*	145.6Å45.7*
G-GT (IU)	0.2Å0.4	1.2Å2.2	1.3Å2.6	0.5Å1.4
Bilirubin (mmol/l)	3.5Å1.5	4.4Å1.7	4.4Å1.8	4.4Å1.3
Creatinine (mmol/l)	60.6Å5.2	54.6Å4.1**	55.8Å3.1*	57.6Å2.5
Urea (mmol/l)	6.8Å0.7	8.2Å1.1**	7.4Å0.7*	6.0Å1.2
Albumin (%)	17.0Å2.3	20.4Å2.6**	15.9Å3.4	13.9Å2.2**
Glucose (mmol/l)	7.0Å1.4	6.6Å0.8	8.3Å8.8*	7.0Å0.9
Sodium (mmol/l)	146.4Å1.4	147.7Å1.9	145.2Å1.4	147.8Å1.3*
Potassium (mmol/l)	6.4Å0.5	5.8Å0.9*	6.1Å0.4	6.2Å0.3
Chloride (mmol/l)	91.8Å4.8	109.5Å8.8***	104.2Å3.8***	96.4Å3.1*
Calcium (mmol/l)	3.48Å0.48	3.08Å0.45	3.37Å0.93	3.19Å0.33
Phosphorus(mmol/l)	3.31Å0.46	2.77Å0.63*	2.42Å0.34***	2.38Å0.25***

Mean Å SD are given. For statistical significances:*=p<0.05; **=p<0.01;
***= p<0.001

Our study at slightly higher maximal doses showed in 90 days decreased weight gains in males at the two highest dose levels and in females at the highest dose although after 28 days no such changes were yet found. This gives a NOEL (No Observed Effect Level) of 50 mg/kg based on decreased weight gain. The haematological changes after 28 days did not show any dose-responsive consistency and cannot be regarded as Endod-related. The changes after 90 days dosing in haematological parameters are not dose-related either. In clinical chemistry parameters, changes were found in some of the liver function tests after 28 day exposures but were not dose related. After 90 days in test values at the doses of 250 mg/kg and 625 mg/kg in both males and females giving a NOEL level of 50 mg/kg. Changes in other parameters were not uniform and did not show any dose-responsiveness.

These studies suggest that the water extract of *Phytolacca dodecandra* is not mutagenic and has very low acute toxicity. Chronic toxicity studies are necessary both in laboratory and in the field before large-scale general use is initiated of Endod powder as a molluscicide to combat bilharzia and to prevent harmful damage caused by molluscles. The signs of subchronic toxicity appeared at considerably lower levels than those of acute toxicity. The data suggest, however, that the toxicity of Endod is general, without a clearcut target organ in this 90-day toxicity study if not regarding stomach as such. A chronic toxicity and carcinogenicity study would give a final answer on the safe doses and means of use of Endod as a molluscicide. The present study supports the promising possibilities of Endod as a molluscicide due to its relative low toxicity vs its effectiveness. Endod might prove to be a potent natural candidate molluscicide against zebra mussels.

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