



Biochemical and Haematological Assessment of *Croton tiglium* Seeds Mixed with Animal Diet in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript

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ABSTRACT

Aim: The present study evaluates the toxic effects of *Croton tiglium* seeds mixed with animal diet on plasma and blood parameters in male albino rats.
Methodology: Eighteen adult albino male rats, of age 6-8 weeks with an average body weight 120-160 grams, were used in this study. After acclimatization period the animals were divided into 3 groups each of 6, and they treated as follow: Group (I): given normal animals and served as control. Group (II) and (III): were given a mixture of animal's diet with *Croton tiglium* crushed seeds at concentrations of 10% or 20% respectively. The experiment was conducted for two weeks. During the experiment, animal's body weight was taken weekly, clinical observations and mortality were also recorded daily. At the end of the experiment, animals were slaughtered and blood samples were collected into bottles containing no anticoagulant the blood samples were allowed to clot and serum was obtained by centrifuging at 1609.92 g for 5 minutes, the clear serum was removed by pipetting and some of biochemical variables were then determined. The other part of

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the blood was collected into Ethylenediaminetetraacetic acid (EDTA) – containing samples bottles for haematological analysis.

Results: Blood glucose levels of rats fed a diet containing *Croton tiglium* seeds show a significant decrease in the concentration of both treated groups compared with control group. Total protein, albumin and globulins in animals in groups who fed with a diet containing 10% and 20% *Croton tiglium* plant seeds were at the same levels of their values in control rats and no significant changes in these parameters compared with the control. Urea showed a significant increase. A significant increase in total cholesterol concentration in group III and non significant increase in group II compared with group 1 at $P \leq 0.05$. No significant change in activity between control and treated animals was occurred in Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) activity, while Aspartate aminotransferase (AST) activity showed significant decrease in group 3 who fed a diet containing 20% Croton seeds and an elevation of about 20% to animals in group II. Haemoglobin (Hb), red blood cells count (RBCs) and Packed cell volume (PCV) showed significant increase values over control when rats fed with a diet containing either 10% or 20% crude *Croton tiglium* plant at $P \leq 0.05$, and significant decrease in Mean corpuscular haemoglobin (MCH). Other parameters, White blood cells count (WBCs), Mean corpuscular haemoglobin concentration (MCHC), and Lymphocytes (LYM) showed no remarkable alteration from control values at either dose treatment.

Conclusion: Croton seeds administration at doses of 10% and 20% have little effect on some haematological indices specially those relating to red blood cell and white blood cells.

Keywords: Biochemical; haematological; croton tiglium; euphorbiaceae; assessment.

1. INTRODUCTION

Croton tiglium L., which locally known as “Habat el – Mulook” is belong to family Euphorbiaceae. It is used in Sudanese medicine, the powder of the seeds is mixed with dates (*Phoenix dactylifera*) as a laxative [1]. Other traditional uses include tumors and cancerous sores. The seeds are source of a commercially available oil (croton oil), used as purgative [2]. There are abundant linoleic acid, oleic acid and eicosenoic acid in methyl–esterified sample of seed oil extracted from *C. tiglium* from China, isoborneol, fenchyl alcohol and phorbol esters were also found in croton seeds [3].

The study was carried out in order to assess the toxic potential of *Croton tiglium* seeds which are of medicinal value, using changes in haematological and biochemical parameters as indices of toxicosis.

2. MATERIALS AND METHODS

2.1 Plants Materials

Croton tiglium seeds obtained from herbalist at Omdurman market, Sudan, during March, 2008. Crushed seeds were used and added at different concentrations of 10% and 20% to the normal animal diet. The plant seeds were identified and authenticated by Prof. Hatil El – Kamali.

2.2 Experimental Animals

Eighteen adult albino male rats, of age 6-8 weeks with an average body weight 120-160 grams, were used in this study. They brought from National Centre for Research (NCR), Medicinal and Aromatic Research Unit, Khartoum, Sudan. All animals housed and reared at the premise of the (NCR) in metal cages for acclimatization period of a week, and maintained under controlled conditions with food and water *ad libitum*.

2.3 Animal's Diet

Animals' diet was prepared by mixing 300 gram meat + 700 gram wheat + salt + water according to the preparation in Medicinal and Aromatic Plants Institute, NCR, Khartoum.

2.4 Kits

Kits used in this work for biochemical investigations were purchased from BioSystem Company agents at Khartoum, BioSystem S.A. Costa Brava 30, Barcelona and Coromatest (Linear Chemicals), Spain.

2.5 Experimental

After acclimatization period, the animals were divided into 3 groups each of 6, and they treated as follow: Group (I): given normal animals and

served as control. Group (II) and (III): were given a mixture of animal's diet with *Croton tiglium* crushed seeds at concentrations of 10% or 20% respectively. The experiment was conducted for two weeks. During the experiment, animal's body weight was taken weekly, clinical observations and mortality were also recorded daily. At the end of the experiment, animals were slaughtered and blood samples were collected into bottles containing no anticoagulant the blood samples were allowed to clot and serum was obtained by centrifuging at 1609.92 g for 5 minutes, the clear serum was removed by pipetting and some of biochemical variables were then determined. The other part of the blood was collected into Ethylenediaminetetraacetic acid (EDTA) – containing samples bottles for haematological analysis.

2.6 Biochemical Investigations

All biochemical investigations were carried out at Research laboratories of Sudan University of Science and Technology, National Health Laboratory (Khartoum), Ibrahim Malik Hospital Lab, and Turkish Hospital Lab. Blood glucose was estimated by the glucose oxidase method according to Trinder [4]; total protein of the sample was estimated by Biuret method, according to Gomall et al. [5]. Albumin in the sample was measured with bromocresol green in acid medium forming a coloured complex that can be measured spectrophotometrically according to Doumas et al. [6]. Urea in the sample originates, by means of coupled reactions. The coloured complex formed can be measured by spectrophotometry, as described by Tabacoo et al. [7].

Alanine aminotransferase (ALT) estimated in the sample according to the decrease of the rate of formation of reduced NAD (NADH) as a result of formation of glutamate and pyruvate. Aspartate aminotransferase (AST) measured by the same principle of Alanine aminotransferase (ALT).

Alkaline phosphatase catalyzes the hydrolysis of 4-nitrophenylphosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, the rate of formation of nitrophenol, proportional to the activity of Alkaline phosphatase (ALP) present in the sample.

The Principle of the total cholesterol method based on that the cholesterol present in the sample gives coloured complex. The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Triglycerides in the sample originates by means of the coupled reactions, the coloured complex that can be measured by photometry using glycerol phosphate oxidase/ peroxidase methods of Bucolo and David, [8].

2.7 Haematological Investigations

Blood samples were analyzed for haematological index at the National health laboratory and Turkish Hospital Lab: White blood cells count (WBCs), Red blood cells count (RBCs), Haemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Platelets (PLT) and Lymphocytes (LYM).

2.8 Statistical Analysis

Results were presented as mean \pm standard deviation (S.D). Data were statistically analyzed using two –way analysis of variance, the differences between plants treated and control group were analyzed using student t-test to determine their level of statistical significance using SPSS commuter program SYSTAT 10.2. Statistical significance was considered at $P \leq 0.5$.

3. RESULTS AND DISCUSSION

3.1 Body Weight Changes

Table (1) represent the body weight results. A decrease of about 6.6% and 18% in body weight in animal of group II and group III respectively was noticed compared with the control. There was a correlation between the loss in animal body weight and the amount of the diet eaten by the animals. The animal body weight loss in both group II and group III was found to be correlated with the amount of diet taken by animals in each group. Diarrhea and the loss of weight might be attributed to affect of crotonic acids. The poisoning symptoms which appeared on rats in group (II) and group (III) maybe due to toxic compound found on Croton seeds. It has long been known that the seeds of *Croton tiglium* contain powerful toxic substances in the oil. The oil contains vesicant and purgative properties. The investigations isolated a principle that was both vesicating and purgative closely related to risinoleic acid to what they gave the name crotonoleic acid [9]. The seeds are poisonous and contain crotonin, taxalbumin. It is a drastic purgative drug; its strong cathartic action begins with irritation in stomach, gripping in intestines,

irritation in intestinal mucous membrane and results in watery motions repeatedly [10].

3.2 Biochemical Analysis

The results of biochemical analysis of blood taken from the three groups under the treatments are shown in Tables 2. Blood glucose levels of rats fed a diet containing *Croton tiglium* seeds show a significant decrease in the concentration of both treated groups compared with control group. Total protein, albumin and globulins in animals in groups who fed with a diet containing 10% and 20% *Croton tiglium* plant seeds were at the same levels of their values in control rats and no significant changes in these parameters compared with the control. Urea showed a significant increase. A significant increase in total cholesterol concentration in group III and non significant increase in group II compared with group 1 at $P \leq 0.05$. No significant change in activity between control and treated animals was

occurred in Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activity, while Aspartate aminotransferase (AST) activity showed significant decrease in group III who fed a diet containing 20% Croton seeds and an elevation of about 20% to animals in group II.

3.3 Haematological Investigations

Haemoglobin (Hb), Red blood cells count (RBCs) and Packed cell volume (PCV) showed significant increase values over control when rats fed with a diet containing either 10% or 20% crude *Croton tiglium* plant at $P \leq 0.05$, and significant decrease in Mean corpuscular haemoglobin (MCH). Other parameters, White blood cells count (WBCs), Mean corpuscular haemoglobin concentration (MCHC), and Lymphocytes (LYM) showed no remarkable alteration from control values at either dose treatment.

Table 1. Average body weight, percentage body weight gain / loss and amount of diet eaten by rats fed different concentration of *Croton tiglium* for 14 days

Treated group	Average body weight (gms)		P-value	% Body weight gain/loss	Amount of diet eaten by group (gms)
	Initial	Final			
Group (I)	85.00±0.00	149.5±20.43	0.001	76% Gain	1985
Group (II)	105.02±4.47	98.72±8.01	0.08	-6.6% Loss	200
Group (III)	90±0.00	73.62±5.16	0.002	-18% Loss	168

Table 2. Biochemical test result of blood of the albino rats

	Group (I) Control (normal animal diet)	Group (II) (animal diet mixed with 10% of plant powder)	Group (III) (animal diet mixed with 20% of plant powder)
Glucose (mg/dl)	97±6.84	80.831±6.2	66.81±2.2
Total Protein (g/dl)	7.120±.34	6.910±.42	7.040±.45
Albumin (g/dl)	3.120±.38	3.630±.48	2.910±.20
Globulins (g/dl)	3.990±.27	3.711.±51	4.120±.71
Urea (mg/dl)	44.884±.20	59.27.±69	70.766±.92
Total cholesterol (mg/dl)	127.1663±2.5	140.66+48.939	162.16±14.87
Triglyceride (mg/dl)	87.52±9.84	48.951±4.478	65.621±6.17
Alanine aminotransferase (ALT) (u/L)	35.2264.±01	28.801±0.75	32.01±3.08
Aspartate aminotransferase (AST) (u/L)	170.8836±.64	179.832±3.66	123.166+30.78
Alkaline phosphatase (ALP) (u/L)	49.1661±7.96	35.101±2.9	45.551±3.212

3.4 Mortality and Clinical Features

Animals in both treated groups (II) and (III) who fed a diet containing 10% and 20% Croton seeds respectively exhibited severe poisoning symptoms, such as depression, diarrhea, and anorexia, when compared with control group. No death occurred among treated rats.

The decrease in glucose levels noticed in the treated animals also might be attributed to affect of crotonic acid. It mentioned that crotonic acid had been extracted from the seeds while it absorptive of glucose from intestine.

Although cholesterol showed slightly but significant increased (in group treated with 20% *Croton tiglium* seeds mixed with the diet) than control value, the levels are within normal blood cholesterol levels.

Assay of selected enzymes activities may help to identify the damage tissues and knowledge of pattern of enzyme changes together with the clinical and other findings are needed if a useful interpretation is to be made.

It is well documented that Aspartate aminotransferase (AST) is present in high concentration in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes while Alanine aminotransferase (ALT) is also found in high concentration in liver and to a lesser extent in skeletal muscles, kidney, and heart (Mayne, 1994). Damage of any of these tissues or organs may increase plasma Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels. It has been reported that liver cells damage is characterized by release of enzymes Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) due to hepatocytes damage, moreover, Aspartate aminotransferase (AST) activity have been reported to increase in certain pathological conditions such as Red blood cells count (RBCs) haemolysis, circulatory failure as a result of chock, hypoxia, myocardial infarction, toxic hepatitis, cirrhosis and haemolysis (due to liver involvement), liver congestion secondary to congestion cardiac failure (Chatterjac and Shind, 1994). In this study slight liver damage and signs of hepatic cells abnormality were seen.

Similarly, Alkaline phosphatase (ALP) is a group of enzyme present in most tissues but in particularly high concentration in the osteoblast

of bone, the cells of the hepatic tract, intestinal wall, renal tubules and placenta. The activity of this enzyme Alkaline phosphatase (ALP) nearly remains unchanged until the end of the experiment, which may indicate that the plant seeds have no profound effect on this enzyme. It has been found that high activities of this enzyme are found in liver disease such as space – occupying lesions e.g. abscess, primarily carcinoma and metastatic carcinoma, xanthomature bilillary cirrhosis, infiltration lesions, like lymphoma, granulomas, and amyloidosis.

Haematological index showed marked alteration in some parameters. A significant increase ($P \leq 0.05$) was noticed in Haemoglobin (Hb), Red blood cells count (RBCs), and Packed cell volume (PCV), and the increase was occurred under both Croton seed doses (10% and 20%). However, Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular volume (MCV) and Lymphocytes (LYM) were not much altered and no significant alteration occurred compared with control. This imply that Croton seed, may affected or increase the incorporation of haemoglobin into red blood cells, and also may have effect on the morphology and the osmotic fragility of red blood cells production, or the Croton seeds may increase the population of red blood cells production from bone marrow. Since the increase of Haemoglobin (Hb), Red blood cells count (RBCs) and Packed cell volume (PCV), observed in this study may suggest that the animals under these treatments were not suffered anemia in which no impairment of red blood cell production occurred.

White blood cells count (WBCs) on the other hand, showed almost no remarkable or significant change from control. This may suggest that Croton seeds of both treatments have no defect or adverse effect on White blood cells count (WBCs), no destruction or impaired production of WBCs occurred, also the stem cells responsible for production of White blood cells count (WBCs) were not affected by the treatment. The Croton seeds treatments did not have significant alteration in platelets compared with control. Normality of White blood cells count (WBCs) at the two treatment without corresponding reduction of platelets suggest that the Croton seeds at these levels of doses have no damaging effect on bone marrow depression, and the bone marrow is known as responsible for the production of red blood cells and white blood cells and platelets.

Table 3. Haematological test result of blood of the albino rats fed different doses of *Croton tiglium* crude plant seeds mixed with animal's diet for 2 weeks

	Group (I) Control (normal animal diet)	Group (II) (animal diet mixed with 10% of plant powder)	Group (III) (animal diet mixed with 20% of plant powder)
Hb (g/dl) Haemoglobin	12.5±1.2	14.3±.46 ^S	13.8±.18 ^S
WBCs x10 ³ u/L White blood cells count	6.2±2.5	7.1±0.9 ^{N.S}	7.1±1.73 ^{N.S}
RBCs x10 ⁶ u/L Red blood cells count	6.6±.83	8.0±.17 ^S	7.7±.59 ^S
PCV (%)	39.3±2.7	44.8±1.6 ^S	47.7±10.9 ^{N.S}
Packed cell volume MCV (fl)	59.9±4.1	55.8±1.3 ^{N.S}	54.6±.9 ^S
Mean corpuscular volume MCH (Pg)	18.9±.6	17.9±.25 ^S	17.4±.30 ^S
Mean corpuscular haemoglobin MCHC (g/dl)	31.7±1.4	31.9±0.4 ^{N.S}	29.9±5.1 ^{N.S}
Mean corpuscular haemoglobin concentration PLT x10 ³ /uL Platelets	399±163	198.5±113.8 ^S	435±213 ^{N.S}
LYM (%) Lymphocytes	67.9±14.3	67.4±5.8 ^{N.S}	61.4±14.4 ^{N.S}

Values within column represent mean ± SD, S = significant at P ≤ 0.05, N.S = not significant at P ≤ 0.05

Crotoncudin have a pharmacological effect was extracted from the *Croton tiglium* [11]. Aqueous extract of *C. tiglium* was exhibit genotoxicity, acute toxicity, anticancer and antioxidant activities [12] and [13]. Cytotoxic phorbol esters were extracted from *C. tiglium* [14]. Therefore, the plant extract need to be evaluated for its long term human health hazards for safe use.

4. CONCLUSION

We may concluded that Croton seeds administration at doses of 10% and 20% have little effect on some haematological indices specially those relating to red blood cell and white blood cells.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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