Cinnamic Acid Activity on Complete Blood Count Tests Against Trichlorfon Residues in Mice

Niba,Kh.Mousa[1]; Jabbar,F. Al-maadhidi[2]; Abrar, N.Mohammd[1]; Eman, H. Qatia[1]; Amal, Ab. Halub[1]; Duha, B. Mohammed[1]; Alaa,D. Kadhum[1]; Azhar,S. Abd-alkharem[1]; Mohamed, Ab. Ayyash[1]; Ishrak, Ab. Ahmed[3]

[1]Environmental-Water Research and Technology Directorate, Ministry of Science and Technology.

[2]Madenat Alelem University College.

[3]The National Center for Drug Control and Research, Ministry of Health.

Abstract:

The current study aimed to evaluate the role of cinnamic acid and its activity on complete blood count (RBC, WBC, HG, HCV, MCH, MCHC and Platelets) and protective effect against the trichlorfon which is a chemical compound that damage hepatic cells and has mutagenic effects. Two concentrations of pure cinnamic acid (30 & 60 mg/kg) were used in the first step to choice the perfect concentration in comparison with negative and positive controls of trichlorfon. The comparison group represent vitamin C. The second step was carried out to understand cinnamic acid mechanism activity towards trichlorfon by used pre-trichlorfon and post – trichlorfon in interaction with perfect concentration of cinnamic acid dose (30 mg / kg).The analysis showed that cinnamic acid removed kinds of anemia ,leukemia,bone marrow failure, hypoxia, cancer chemotherapy, hemolytic anemia and hormone erythropoietin from kidney failure in post-trichlorfon than pre-trichlorfon perfectly. Therefore, cinnamic acid has cure ability and removed trichlorfon damage and it can be given to patient whom used trichlorfon in transplanting body part surgeries to a void refused the part for 6 days after transplanting surgeries.

Keywords: Cinnamic acid, Trichlorfon, Complete Blood Count, Mice.

فعالية حامض السينامك على اختبار مجاميع الدم تجاه بقايا مبيد الترايكلورفان في الفئران البيض

نبال خليل موسى[1]،جبار فرحان المعا

ضيدي[2] ،ابرار ناطق محمد [1]،ايمان هندي كاطع [1]،امل عبد النبي حالوب[1]،ضحى بهاء محمد[1]،الاء داغر كاظم [1]،ازهر سالم عبد الكريم[1]،محمد عبود عياش[1]،إشراق عبد الأمير احمد [3]

1. دائرة تكنولوجيا وبحوث البيئة والمياه– وزارة العلوم والتكنولوجيا.بغداد–العراق

2. كلية مدينة العلم الجامعة.

3. المركز الوطني للرقابة والبحوث الدوائية،وزارة الصحة.بغداد–العراق.

الخلاصة

هدفت الدراسة تقييم ومعرفة مدى فعالية حامض السينامك على نشاط تعداد الدم الكامل (RBC,WBC,HG,HCV,MCH,MCHC and Platelets)وازالة الاضرار التي تنجم عن بقايامبيد الترايكلورفان والذي يعد مركب كيمائي يسبب تلف خلايا الكبد .انتخب تركيزين من حامض السينامك (30 & 60 ملغم /كغم)في المرحلة الاولى لاختيار التركيز الامثل بالمقا نة مع كل من السيطرة السالبة ، السيطرة الموجبة للسايتوكسان ومجموعة المقارنة لفيتامين سي اما الخطوة الثانية فقد تضمنت دراسة الية عمل حامض السينامك تجاه السايتوكسان بطريقة قبل– السايتوكسان وبعد السايتوكسان بالتداخل مع التركيز الامثل للحامض (30 ملغم /كغم)واظهرت التحاليل ان لحامض السينامك اثر في فقر الدم ، سرطان الدم ،الفشل النخاعي ،نقص الاوكسجين ،العلاج الكيميائي للسرطان ،فقر الدم الانحلالي والفشل الكلوي في مرحلة مابعدالمبيد تماما لهذا فان لحامض السينامك صفة علاجية واعطائه لمدة 6 ايام لازالة ضرر المبيد.

Introduction

In the last decades, the development industry and growing human action in discovery many chemical components that use in difference sides, some of them, pesticides industry, drugs and nutrition industry, which increasing environmental pollution and reflected on the health of human body [1]. These compounds have the ability to cause damage in different tissues and systems of human body, whether the expose directly or indirect albeit little concentrations because most of them can accumulation in cells and tissue, then conversion to derivatives rich in electrons [2]. The frequently cell exposed execute to making changes in DNA that finally caused cancer. The statistics assure that near 85% from mutation components are carcinogenic components [3].

Trichlorfon is an organophosphate insecticide used for control cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leaf miners and leaf-hoppers [4]. It is applied to vegetable, fruit and field crops; livestock; ornamental and forestry plantings; in agricultural premises and domestic settings; in greenhouses, and for control of parasites of fish in designated aquatic environments [5]. It is also used for treating domestic animals for control of internal parasite [6,7]. It is available in dust, emulsifiable concentrate, granular, fly bait, and soluble powder formulations [8].

Trichlorfon is a selective insecticide, meaning that it kills selected insects, but spares many or most other organisms. Trichlorfon is toxic to target insects through direct applications and by ingestion. In other words, it works both by contact and stomach poison action [5]. Trichlorfon is one of organophosphates family of insecticide. These chemicals act by interfering with an essential nervous system enzyme, cholinesterase.

Plants have the role in inhibition cancered components and protective effects. The primary studies show that the cancer less rate in societies that vegetarianism habitat in compare with societies least used plants in their daily food [9]. The natural vegetarianism components, nutrition and non–nutrition due to their important role in keeper the human health and improvement his life.

The bioaviabilities of plant polyphenol and their ability to inhibit and prevent tumor after entering blood circulation and absorbing by bowel belong to their affection on protein or control factors and the role in repairing cells [10], in addition to motivate immunology system,increasing natural killer cells (NK) and effect on the enzymes which responsible for

process and complete the cell cycle by hyper-expression arrangement [11].

Complete blood count (CBC) gives important information about kinds and numbers of cells in the blood, especially red blood cells (RBCs), white blood cells (WBCs) and platelets. A complete blood count (CBC) helps in diagnosing conditions, such as anemia, infection, and many other disorders. To quantify the toxicity of trichlorfon, the next important step is to assess the various hematological parameters such as hematocrit (HCT), hemoglobin (HB), red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) count [12].

In the last years, the consumption of cinnamon increasing widely in many countries, because of the ability to prevent some kinds of cancer due to their components and its effect on mutation and cancer by interacting in metabolism reactions and bio-pathways of these components in body [13]. Searcher interesting increased in finding methods express the components characters of cinnamon such as cinnamic acid and invention of activity to know if they are oxidant or antioxidant, cancered or protective. Many of organizations and science corporations recommended to apply and use the biosystem to detect the toxicity of manufacture components or natural whether are *in vitro* or *in vivo*, some of them the mammalian systems that use the animals of laboratory mice with all different organisms, blood cells and bone marrow in additional the sexual cells like spermatic.

The study aimed to evaluate the role of cinnamic acid in removing the trichlorfon effect that reflect on complete blood count and documenting the ability of cinnamic acid in removed bone marrow failure and leukemia due to trichlorfon, which had no reported before.

Materials and Methods

Material

- Phosphate Buffer Solution (PBS) [13].
- Colchicine solution: 1 mg of colchicines (one tablet) was dissolved in 1 ml sterile distilled water. The solution was used immediately after preparing (2.5 to 3 hours post the timed of preparation) [15].

Doses

Two doses from the pure cinnamic acid which were (30, 60) mg/mice weight and vitamin C (180 mg/kg) [16] as comparative groups and trichlorfon compound in (50 mg/kg) [13] as a positive control and the PBS as a negative control [18].

The experiment

To study the oxidant effect and the antioxidant in laboratory animals:

- 1 ml of cinnamic acid solution was given orally by syringe.
- The Trichlorfon solution was injected Intraperitonially [14].

Fifty –five mice, weighing 20–25 g were divided into three groups: choice perfect cinnamic acid concentration (negative control (PBS),positive control (Trichlorfon) comparative group vitamin C. and two cinnamic acid concentration (30.60) mg/kg , pre–Trichlorfon and post –Trichlorfon figure 1.

Collection of Blood and Serum

The blood was collected from the heart of mice (*Mus musculus*) and collected for experiment using Heparin (10 units/ml) as the anticoagulant from all animals on the seventh day after experiment [19].

Hematological analysis

The hematological autoanalyzer (Orphee Mythic 22 Hematological Analyzer; Diamond Diagnostic; USA) was used to determine different hematological parameters, such as Red Blood Cells (RBC), White Blood Cells (WBCs), Hemoglobin (HB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red blood cell Distribution Width (RDW), Platelet Distribution Width (PDW)%, Platelet crit (PCT)% and Platelets (PLTs) [20].

Statistical analysis

The statistical analysis has been used to study the effects of treatments in different trails. The least significant difference (LSD) test was used to signify a comparison between the means [21].

Results and Discussion

Perfect cinnamic acid concentration compared with Trichlorfon.

The effect of the perfect concentration of cinnamic acid extract (30 mg/kg) for 7 days treatment in mice were showed lack of influence, other denoted like changes in color and thickness of mice hair, eyes shape, change in weight, change in liver function enzymes and the antioxidant enzyme, while concentration (60 mg/kg) showed changes in color of mice hair as it became light yellow, lowing in thickness and losing hair in some parts of the body, beside that changes in the eyes shape and lose their bright, in addition to increase in LFTs and antioxidant enzymes [19] when compared with the negative, positive treated and Vit.C because high dose of vitamins and minerals can be toxic [20].

The concentration (30 mg/kg) of cinnamic acid extract showed excellent results in increasing weight table 1, active in moving, increasing the ability to eat more, increasing in mice hair thickness to be more

white than the normal and the eyes were bright with no changes in shape. In the other side the LFTs and the antioxidant were a good results (when compared with the negative, positive treated and Vit.C). Trichlorfon was injected Intraperitonially member because gulping caused losing within 3-12 hours [21].

Trichlorfon was caused inactive in moving of mice, losing the hair in the back, legs, shoulders [14], because trichlorfon is a chemotherapy, which makes cells grow slow or block the cell growth [21]. Cytochrome P-450 was group of enzymes in endoplasmic reticulum known as the most important family of metabolizing enzymes in liver and the terminal electron transport chain of oxidase [22].

The main effect of trichlorfon by metabolizing several pathways. The two major reactions include: hydrolysis of the methoxylmoiety [23] with the methyl group being incorporated byalkylation or methyl transfer into proteins in liver and various organs [24] and hydrolysis of the phosphonate (P-C) bond [25] yielding trichloroethanol which is subsequently conjugated. Others have suggested that conjugated metabolites from rabbits contain molecule that has an altered trichloroethyl moiety and an intact phosphorus atom. This alteration product has not been further defined. In most instances the metabolism in plants and animals appears to follow the same route [26].

The lymphocytes (WBC) represent the first line against the different infections in body [27]. Trichlorfon (medicine that can decrease the immune response) [21] has been showed lower than normal white blood cell counts which called leucopenia, which may be due to autoimmune disease, bone marrow failure (4) tumor, fibrosis and disease of liver [14,19], when compared with negative control (PBS) and vitamin C., while the cinnamic acid (60mg /kg) showed increased than normal of numbers of WBCs, which called leukocytosis when compared among PBS vitamin C and trichlorfon treatment, results from leukemia and tissue damage [28].

Red blood count showed decreased in both trichlorfon and cinnamic acid (60 mg/kg) when compared with PBS and vitamin C. The reduce in RBCs count means increased destruction of red blood cells or layses of red blood cells. Lack of iron, vitamin B12, folic acid in diet as well as certain chronic diseases [19]. Lower the number of red blood cells produced by bone marrow failure, chronic kidney disease, hemolysis, leukemia, long term infections (hepatitis) and other blood cancers due to tumor or fibrosis [29]. trichlorfon caused red blood cells to break down earlier than normal which called immune hemolytic anemia secondary to drugs. In the same time cinnamic acid (30 mg /kg) showed increased with normal when

compared with vitamin C and BPS and trichlorfon.

Hematocrit (HCT) is a blood measures the percentage of whole blood volume that is made up of red blood cells. This measured depend on the number of red blood cells and the size of RBCs. Trichlorfon figure 2 showed lower than normal in hematocrite may due to anemia, bleeding, overhydration, nutrition deficiencies of iron, floate, vitamin B12, B6, malnutrition, destruction of red blood cells [30] in compared with PBS and vitamin C. Cinnamic acid (60 mg/kg) showed high hematocrite may be due to congenital heart diseases, corpulmonale, dehydration, erthyocytosis, low blood oxygen levels (hypixia) [30], while cinnamic acid (30 mg/kg) showed normal increased incompared with PBS and vitamin.

Hemoglobin is a protein in red blood cells that carried oxygen [30]. In figure 2 trichlorfon showed lower than normal may be due to various types of anemia such as chronic disease and leukemia [31], while cinnamic acid (60 mg/kg) showed higher than normal may be due to heart diseases, erthyocytosis and hypoxia but cinnamic acid (30 mg/kg) showed increased with normal when compared with each PBS and vitamin C.

Red blood cells (RBCs) are part of the complete blood count (CBC) test. They are used to help diagnose the cause of anemia a condition in which there are too few red blood cells. RBCs include: Average red blood cell size (MCV), hemoglobin amount red blood cell (MCH) and MCHC referred to the amount of hemoglobin relation to the size of the cell (hemoglobin concentration)per red blood cell. In figure 2 trichlorfon showed decreased in MCV which means microcytic anemia [30] and increased in MCH mean normochronic anemia and decreased in MCHC mean different types of anemia when each one compared with PBS and vitamin C. Microcytic anemia / normochronic anemia results from adeficiency of the hormone erthyropoietin from kidney failure [31]while cinnamic acid (30 mg / kg) showed normal levels in MCH, MCV and MCHC when compared with BPS, vitamin C and trichlorfon.

Platelets count helps the blood clot. They are smaller than red and white blood cells. In figure 2 trichlorfon showed lower than normal levels of platelets numbers when has been compared with PBS and vitamin C which due to cancer chemotherapy [21], hemolytic anemia hyperspleism, leukemia and heart valve [30], while cinnamic acid (30mg/kg) showed increased with normal numbers when has been compared with PBS ,vitamin C and trichlorfon treatment.

Interaction Trichlorfon and Perfect concentration of cinnamic acid (30 mg/kg)

The interaction among trichlorfon and PBS, vitamin C and perfect concentration of cinnamic acid dose (30 mg/kg) in post-trichlorfon showed in figure 3 the best results in comparison with pre-trichlorfon treatment. Cinnamic acid dose (30mg /kg) showed high reaction in remove trichlorfon effects and increased each of WBC, RBC, MCH, MCHC, MCV, HCT, Hb and platelets count, mechanisms of cinnamic acid to repair removed were:

- Phenolic hydroxyl groups are good hydrogen donors [29]. Hydrogen donating antioxidants can react with react oxygen and reactive nitrogen species and breaks the cycle of generation of new radicals [31].
- Following interaction with the initial reactive species, a radical form of the antioxidant was produced and had a greater chemical stability than the initial radical [32].
- Interaction of phenol hydroxyl groups with π-electrons of benzene ring gave molecules with special properties, the ability to generate free radicals where stabilized by delocalization. Formation of these long-lived free radicals is able to modify radical-mediated oxidation processes [31].
- Antioxidant capacity of phenolic compounds is also attributed to ability chelate metal ions involved in production of free radicals. However, phenolic compounds can acts as pro-oxidants by chelating metals in manner that maintains or increases their catalytic activity or by reducing metals, thus increasing their ability to form free radicals [32].
- Hydrophobic benzenoid rings and hydrogen bonding potential of phenolic hydroxyl groups interact with protein and gave cinnamic acid capacity to inhibit some enzymes involved in radical generation [31, 32].
- Avoid and prevent hydroxyl radical as a product of hydrogen peroxide and gave the first spark for start the chemical interaction such as lipid peroxidation [32].
- Avoid or prevent or repair oxidation of DNA and protein, which depend on the hydroxyl groups of cinnamic acid [33, 34].
- Cinnamic acid was suppressed hepatic fibrosis and protected liver against damage [35].
- Cinnamic acid has anti-hyperlipidemic action [36].

- Release of inflammatory mediators such as cytokines, histamine, prostaglandins and leukotrenes to protect hepatocyte [36].

Conclusion

1. Cinnamic acid has no side effect in dose 30 mg/kg.
2. Leukopenia, bone marrow failure and fibrosis which reflected from decreased WBC due to trichlorfon removed by pure cinnamic acid in post-trichlorfon perfectly.
3. All kinds of anemia which reflected from hemoglobin (Hb) analysis removed when used cinnamic acid dose (30 mg/kg) perfectly in post-trichlorfon treatment.
4. Anemia bleeding, heart disease and low blood oxygen levels (hypoxia), which reflect from hematocrit (HCT) removed due to cinnamic acid dose (30 mg/kg) perfectly in post-trichlorfon treatment.
5. The ratio between MCHC/MCH showed microcytic anemia /normochronic anemia due to deficiency of hormone erythropoietin from kidney failure which reflected from trichlorfon treatment in each perfect concentration pre-trichlorfon and post-trichlorfon and removed perfectly in post-trichlorfon due to cinnamic acid dose (30mg/kg).
6. The ratio of ratio platelets count (RWD/CV, RDW/SD) showed increased in normal could remove (cancer chemotherapy, hemolytic anemia and leukemia) trichlorfon effects perfectly by cinnamic acid dose (30 mg/kg) in post-trichlorfon treatment.

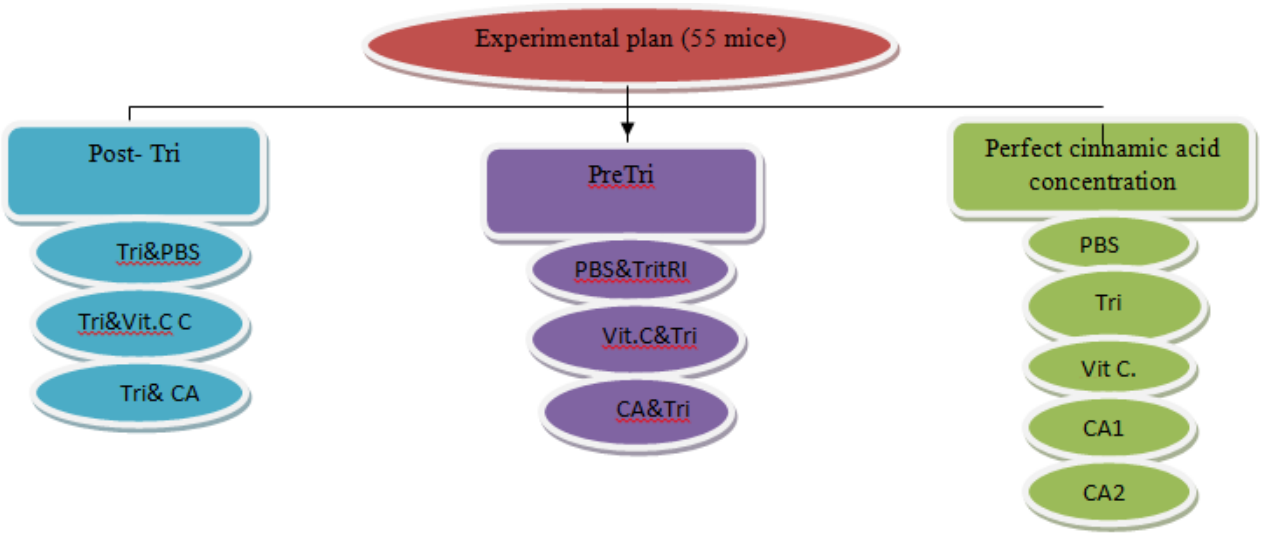

Figure 1. Experimental plan. *Tri =Trichlorfon, CA= cinnamic acid,PBS=phosphate buffer solution, Vit C=vitamin C.

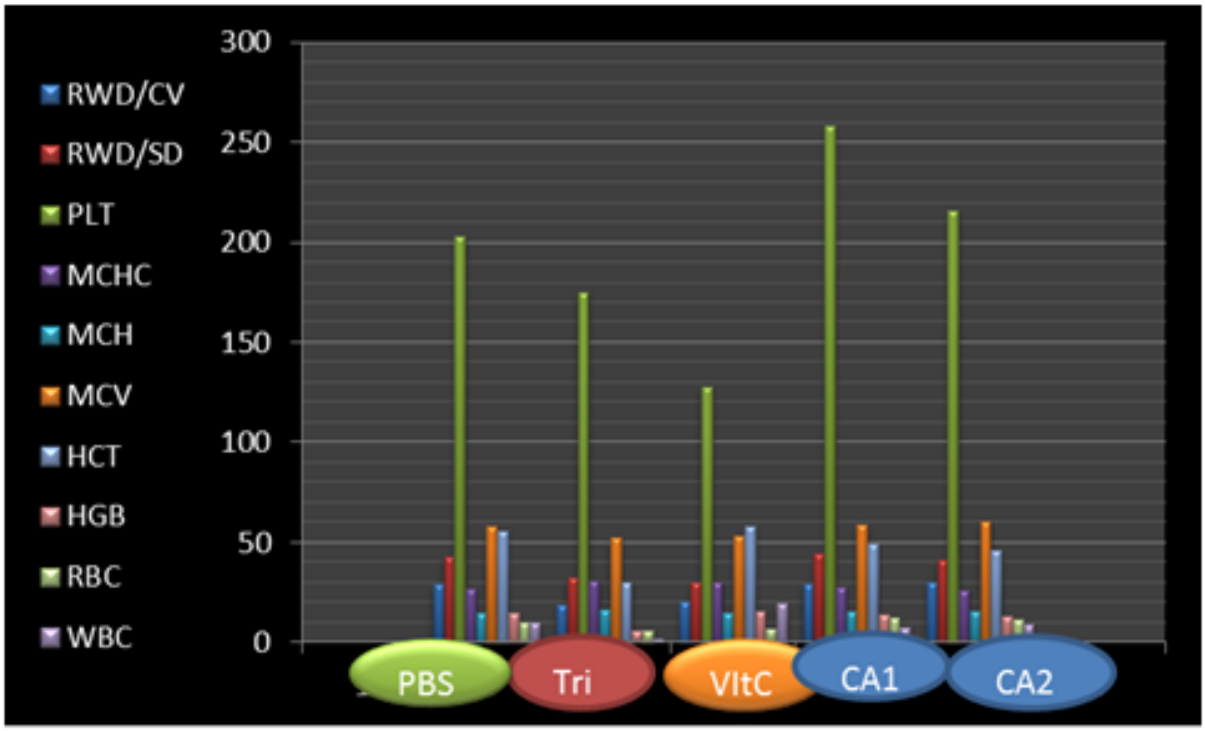

Figure 2. Complete blood count and perfect concentration of cinnamic acid. PBS: phosphate buffer solution, Tri: trichlorfon, CA: CA1: cinnamic acid dose (60 mg /kg), CA2: cinnamic acid dose (30mg / kg).

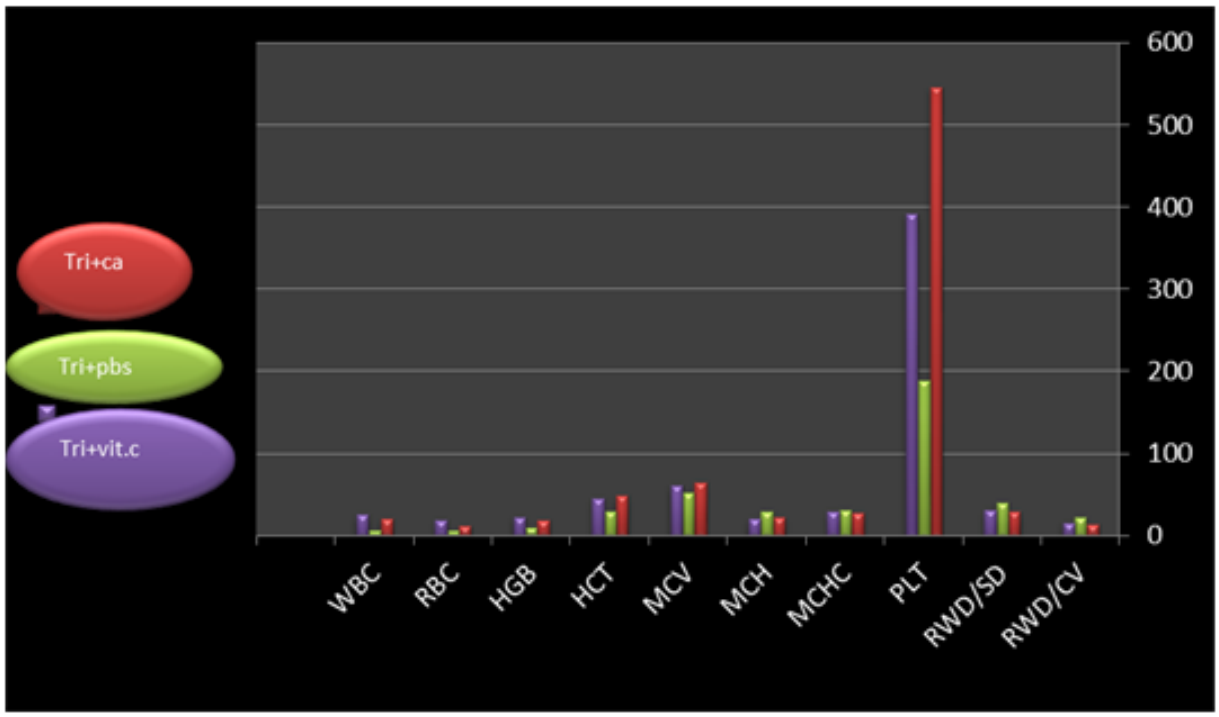

Figure 3. Complete blood cell and post-trichlorfon treatment. PBS: phosphate buffer solution, Tri: trichlorfon, CA: CA1: cinnamic acid dose (60 mg/kg), CA2: cinnamic acid dose (30 mg/kg).

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