

Complete Blood Count and Cinnamic acid activity against Cytoxan in albino mice.

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Abstract:

The aim of study to evaluated cinnamic acid and its activity on complete blood count(RBC,WBC,HG,HCV,MCH,MCHC and Plat.)and removed the cytoxan damage which caused bone marrow failure and leukemia and other that due to linked the cytoxan in 7- nitrogen of guanine based of DNA that lead to dead cells. Two concentration from pure cinnamic acid (5.6, 2.8 mg / mice weight) in first step to choice the perfect concentration in comparison with each negative control ,positive control of cytoxan and the comparison group represent vitamin C. The second step to understand cinnamic acid mechanism activity towards cytoxan by used pre- cytoxan and post – cytoxan in interaction with perfect concentration of cinnamic acid dose (2.8 mg/mice weight).The analysis showed that cinnamic acid removed each kinds of anemia ,leukemia, bone marrow failure, hypoxia, cancer chemotherapy, hemolytic anemia and hormone erythropoietin from kidney failure in post-cytoxan than pre-cytoxan perfectly ,therefore, cinnamic acid has cure ability and removed cytoxan damage and can give to patient whom used cytoxan in transplanting body part surgeries to a void refused the part for 6 days after transplanting surgeries.

Key words: Cinnamic acid, Cytoxan, Complete Blood Count, Mice.

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 the 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 endly caused cancer. The statistics assure that near 85% from

mutation components are cancered components [3].

The effect of Cytoxan toxicity on liver is due to activation of aldophosphamide, is alkyating antineoplastic and immunosuppressive agent that must be activated in the liver, it can cause sterility, birth defects, mutation and cancer. It's one of the many imported components the searchers whipped before the last half century, found assignment mechanisms and basic to damage tissue contrast to lots mechanisms from chemical components, some of them are metabolisms action, mechanism free radical and per oxidation lipids operation, equilibrate trouble of calcium rates in tissues and the indicators about damages [4].

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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 [5]. The natural vegetarianism components, nutrition and non-nutrition due to their important role in keeper the human health and improvement his life.

The bioavailabilities of plant polyphenol and their ability to inhibit and prevent tumor after entering blood circulation and absorbing by bowel belong to their effect on protein or control factors and the role in repairing cells [6], in addition to motivate immunology system, increasing natural killer cells (NK) and effect on the enzymes which responsible of process and complete the cell cycle by hyper-expression arrangement [7].

A 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 CBC(Complete Blood Count) helps in diagnosing conditions, such as anemia, infection, and many other disorders. To quantify the toxicity of CYP(cytoxan), 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) counts[8].

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[9]. The searcher interesting increased in finding methods express the components characters of

Cinnamon such as cinnamic acid and invention of the 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 *invitro* or *invivo*, 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 aim of study t evaluated cinnamic acid in removing the cytoxan effect that reflect on complet blood count and documented the ability of cinnamic acid in removed bone marrow failure and leukemia due to cytoxan which had no reported before.

Material and Methods:

Material:Phosphate Buffer Solution(PBS) (9).Colchicine Solution : Colchicine1mg (one tablet)and sterile distilled water 1ml .The solution was used immediately after preparing 2.5 to 3 hours[11].

Doses: Two doses from the pure cinnamic acid (Riedel-de Haën company)which are(5.6,2.8) mg/ mice weight and vitamin C(180 mg/ kg) [12](as comparative groups and cytoxan compound in (50 mg/ kg) [13] as a positive control and the PBS as a negative control[14].

The experiment: Two concentration from pure cinnamic acid (5.6,2.8) mg/ Kg ,the concentration a count depended on the mouse weight .The experiment contains 40 mice divided in to 5 groups of 8 mice each (16 mice gulped with the two cinnamic acid concentration (5.6,2.8) mg/ mice weight ,8 mice gulped with PBS and depended as a negative control ,8 mice injected with cytoxan compound and depended as a positive control ,8 mice gulped with

vitamin C and depended as a comparative groups and from the two control) and the comparative groups can gain primary idea about the suitable concentration to cinnamic acid ,then study the before- cytoxan and post – cytoxan to understand cinnamic acid mechanisms .

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[15].

Hematological analysis: An hematological autoanalyzer (Orphee Mythic 22 Hematological Analyzer; Diamond Diagnostic; USA) was used to determine different hematological parameters, such as Red Blood Cells (RBC), WhiteBlood 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)%, Plateletcrit (PCT)% and Platelets (PLTs)[16].

Statistical analysis :The statistical analysis is done to get the means \pm SE and test the different significant among the means by using test [17] then differences among the means in interaction experiments were compared between the Vitamin C, cinnamic extract and the cytoxan by using T- test [18].

Results and Discussion:

Cytoxan results and pure cinnamic acid treat *in vivo* (7 days).

The effect of the perfect concentrated of cinnamic acid extract (2.8mg / mice weight) 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 (5.6mg/mice weight) showed lowing in weight and changes in color of mice hair which became light yellow, lowing in thickness and losing hair in some parts of the body, beside that changes in the eyes shape and lose there bright, other side, showed increasing in the LFTs and the 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 (2.8mg/mice weight) of cinnamic acid extract showed excellent results in increasing weight tab(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). Cyclophosphamide was injected in intraperitoneal member because gulping caused losing between (3-12) hours [21].

Cytoxan was caused inactive in moving of mice, losing the hair in the back, legs, shoulders and lowing in LFTs and the antioxidant enzymes[14], because cyclophosphamide was a chemotherapy drug, work by slowing or stopping cell growth when the alkyl group from the cyclophosphamide attaches guanine base in the 7 nitrogen at one of the imidazole ring and stopping the cell divided [21]. Cytochrome P-450 was a 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 cytoxan by metabolizing the phosphoramidate mustard in the cell which have low of aldehyde dehydrogenase (ALDH). Phosphoramidate mustard forms DNA cross links between inter strand cross linkages within intra strand cross linkages at guanine N-7 position with irreversible and leads to dead the cell. Dead cell by cell caused hyper acute liver failure [23].

The lymphocytes (WBC) represent the first line against the different infections in body (24). Cytoxan (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 (56mg/kg) showed increased than normal of numbers of WBCs which called leukocytosis when compared among PBS vitamin C and cytoxan treatment, results from leukemia and tissue damage [25].

Red Blood Count or hematocrit showed decreased in both cytoxan and cinnamic acid (5.6 mg/ mice weight) when compared with PBS and vitamin C. The reduce in RBCs count means increased destruction of red blood cells or lyses 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 [26]. cytoxan caused red blood cells to break down earlier than normal which called immune hemolytic anemia secondary

to drugs. In the same time cinnamic acid (2.8 mg /mice weight) showed increased with normal when compared with vitamin C and PBS and cytoxan.

Hematocrit (HCT) is a blood measure 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. Cytoxan fig (1) showed lower than normal in hematocrite may be due to anemia, bleeding, overhydration, nutrition deficiencies of iron, folate, vitamin B12, B6, malnutrition, destruction of red blood cells [27] in compared with PBS and vitamin C. Cinnamic acid (5.6 mg/mice weight) showed high hematocrite may be due to congenital heart diseases, cor pulmonale, dehydration, erythrocytosis, low blood oxygen levels (hypoxia) [27], while cinnamic acid showed normal increased in compared with PBS and vitamin.

Hemoglobin is a protein in red blood cells that carried oxygen [27]. In fig (1) cytoxan showed lower than normal may be due to various types of anemia such as chronic disease and leukemia [28], while cinnamic acid (5.6 mg / mice weight) showed higher than normal may be due to heart diseases, erythrocytosis and hypoxia but cinnamic acid (2.8 mg / mice weight) showed increased with normal when compared with each PBS and vitamin C.

Red Blood Cells (RBCs) induces 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 fig (1) cytoxan showed decreased in MCV

which means microcytic anemia [27, 28] 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 a deficiency of the hormone erythropoietin from kidney failure [28] while cinnamic acid (2.8 mg / mice weight) showed normal levels in MCH, MCV and MCHC when compared with PBS, vitamin C and cytoxan.

Platelets count help the blood clot. They are smaller than red and white blood cells. In fig (1) cytoxan 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, hypersplenism, leukemia, and heart valve [27] while cinnamic acid (2.8 mg / mice weight) showed increased with normal numbers when has been compared with PBS, vitamin C and cytoxan treatment.

Interaction between Cytoxan and Perfect concentration of cinnamic acid (2.8 mg/mice weight).

The interaction among cytoxan and PBS, vitamin C and perfect concentration of cinnamic acid dose (2.8 mg / mice weight) in post-cytoxan showed in fig(2) the best results in comparison with pre-cytoxan treatment. Cinnamic acid dose (2.8 mg / mice weight) showed high reaction in remove cytoxan effects and increased each of WBC, RBC, MCH, MCHC, MCV, HCT, HG and platelets count, mechanisms of cinnamic acid to repair removed were:

1. 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 [29].
2. Avoid or prevent or repair oxidation of DNA and protein, which depend

on the hydroxyl groups of cinnamic acid [30,31].

3. Cinnamic acid was suppressed hepatic fibrosis and protected liver against damage [32].
4. Cinnamic acid have anti-hyperlipidemic action [33].
5. Release of inflammatory mediators such as cytokines, histamine, prostaglandins and leukotrienes to protect hepatocyte [33].
6. The liver cytochrome p-450 system converts cyclophosphamide to 4-hydroxycyclophosphamide, which is in equilibrium with aldophosphamide. phosphoramidate mustard and acrolein were yielded from cleavage aldophosphamide. These two compounds are highly cytotoxic. Cyclophosphamide is uncommon hepatic toxin and its effect was due to an idiosyncratic reaction [34].
7. Cinnamic acid has kept liver enzyme in normal level such as ALP when increased reflected on bone marrow, AST when increased more than normal level reflected on red cell and cardiac when compared with vitamin C. [19]

Conclusion:

1. Cinnamic acid has no side effect in dose 2.8 mg / mice weight.
2. Leukopenia, bone marrow failure and fibrosis which reflected from decreased WBC due to cytoxan removed by pure cinnamic acid in post-cytoxan perfectly.
3. All kinds of anemia which reflected from hemoglobin (HG) analysis removed when used cinnamic acid dose (2.8 mg / mice weight) perfectly in post-cytoxan treatment.
4. Anemia, bleeding, heart disease and low blood oxygen levels (hypoxia) which reflect from hematocrit (HCT) removed due to cinnamic acid dose (2.8 mg / mice weight) perfectly in post-cytoxan treatment.

5.The ratio between MCHC /MCH showed microcytic anemia/normochronic anemia due to deficiency of hormone erythropoietin from kidney failure which reflected from cytoxan treatment in each perfect concentration pre-cytoxan and post-cytoxan and removed perfectly in post-

cytoxan due to cinnamic acid dose (2.8 mg/mice weight).

6.The ratio of ratio platelets count (RWD/CV,RDW/SD)showed increased in normal could removed (cancer chemotherapy ,hemolytic anemia and leukemia)cytoxan effects perfectly by cinnamic acid dose(2.8 mg /mice weight) in post-cytoxan treatment.

Table 1: Different in mice weight means treated for 7 days.

Treat	Negative group (PBS)	Comparative group Vit.C (180mg/Kg)	Positive group Cyclophosphamide (50 mg /Kg)	Cinnamic acid (5.6mg/mice)	Cinnamic acid (2.8 mg /mice)
Mean ±SE (gram)					
The mouse weight before treating	25.07±1.17	26.7±0.20	29.65±0.30	28.55±0.35	26.7±0.24
The mouse weight after treating	29.75±0.35	26.99±0.49	21.15±2.49	29.45±0.75	32.26±0.44
Weight deferent	4.68±0.002	0.29±0.03	-8.5±0.32	0.9±0.52	5.56±0.33

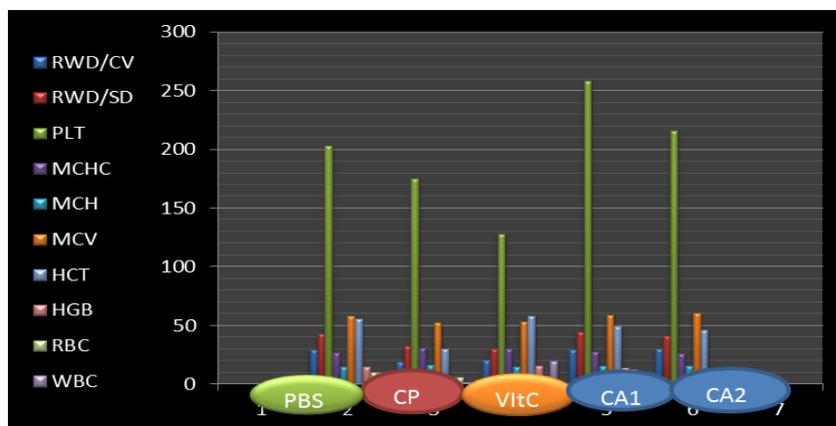


Fig (1)Complete blood count and perfect concentration of cinnamic acid. PBS:phosphate buffer solution,CP:cytoxan,CA:CA1:cinnamic acid dose (5.6 mg /mice),CA2:cinnamic acid dose(2.8mg / mice).

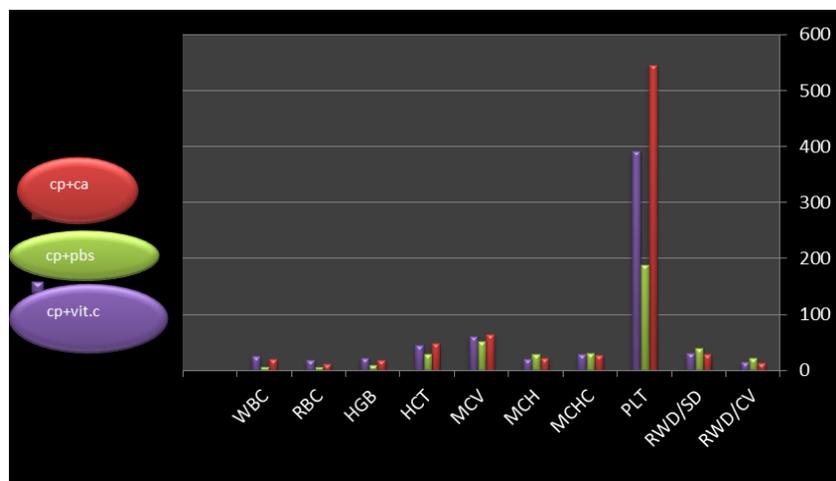


Fig (2) complet blood cell and post-cytoxan treatment. PBS: phosphate buffer solution,CP:cytoxan,CA:CA1:cinnamic acid dose (5.6 mg /mice),CA2:cinnamic acid dose(2.8mg / mice).

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تعداد الدم الكامل وفعالية حامض السيناميك تجاه الساييتوكسان في الفئران المختبرية

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الخلاصة:

اجريت الدراسة لاجل تقييم ومعرفة مدى فعالية حامض السيناميك على نشاط تعداد الدم الكامل (RBC, WBC, HG, HCV, MCH, MCHC and Plat) وازالة الاضرار التي تنجم عن عقار الساييتوكسان المسبب فشل النخاع العظمي وسرطان الدم وغيرها من الاضرار والتي تنجم من ارتباط الساييتوكسان عند جزيئة السابعة من قاعدة الكوانين لحامض الدنا المؤدي الى موت الخلايا. انتخب تركيزين من حامض السيناميك (5.6 ، 2.8 ملغم / من وزن الفارة) في المرحلة الاولى لاختيار التركيز الامثل بالمقارنة مع كل من السيطرة السالبة ، السيطرة الموجبة للساييتوكسان ومجموعة المقارنة لفيتامين سي اما الخطوة الثانية فقد تضمنت دراسة الية عمل حامض السيناميك تجاه الساييتوكسان بطريقة قبل- الساييتوكسان وبعد الساييتوكسان بالتداخل مع التركيز الامثل للحامض (2.8 ملغم / من وزن الفارة) واطهرت التحاليل ان لحامض السيناميك ازالة انواع فقر الدم ، سرطان الدم فشل النخاعي ، نقص الاوكسجين ، العلاج الكيميائي للسرطان ، فقر الدم الانحلالي والفشل الكلوي في مرحلة ما بعد الساييتوكسان تماما لهذا فان لحامض السيناميك صفة علاجية تجاه عقار الساييتوكسان الذي يعطى في عمليات زرع واستبدال الاعضاء كجزء من منع رفض العضو المزروع حيث يمكن اعطائه لمدة 6 ايام بعد اجراء العملية وازالة ضرر العقار.