Cinnamic acid activity against Trichlorfon pesticide toxicity and liver function enzymes in mice.

فعالية حامض السينناميك تجاه سمية مبيد الترايكلورفان وانزيمات وظائف الكبد في الفئران المختبرية

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Abstract

The study was carried out to determine hepatotoxicity and hepatoprotective effects of cinnamic acid in comparison with vitamin C against the mutagenic pesticide influence of trichlorfon, which is a chemical compound that damages hepatic cells and has mutagenic effects. The effect was studied in mammalian system in mice depended on evaluating the enzymatic activity of three hepatic enzymes: Alanine Transaminase(ALT), Aspartate Transaminase (AST) and Alkaline Phosphate (ALP).Two concentrations of pure cinnamic acid (60,30) mg/body weight were tested to choose the suitable concentration which compared with negative control, positive control and comparative group of Vitamin C. In order to use in the interaction experiments, included two types of treatments pre-trichlorfon and post-trichlorfon in order to determine the mechanisms of the pure cinnamic acid, showed no toxic and hepatotoxicity influence in biological system and it showed highly performance in prevention or reduction in hepatotoxicity of trichlorfon. Cinnamic acid increased the ALT, AST and ALP enzymes with normal levels especially with dose of 30 mg/body weight. The positive effect of cinnamic acid was higher when used as post-trichlorfon treatments and to less extent in pre-trichlorfon treatments, therefore, cinnamic acid can be considered as a cure hepatocytes from acute liver damage also work protective to cardiac, skeletal muscle and placental tissue protective.

Keyword: Cinnamic acid, Trichlorfon, Liver Function Enzymes, mice.

المستخلص

أجريت الدراسة للكشف عن التأثير السمي لحامض السينناميك النقي cinnamic acid للكبد ومقارنته بفيتامين C تجاه مبيد الترايكلوروفان Trichlorfon، الذي يعد مركب كيمائي تالف لخلايا الكبد وذلك باستخدام نظام اللبائن في الجرذ وبالاعتماد على تقييم ثلاثة من إنزيمات وظائف الكبد: Alanine Transaminase (ALT), AspartateTransaminase (AST) Alkaline Phosphate (ALP) . استخدم جرعتين لحامض السينناميك النقي 60،30ملغم /من وزن الحيوان وكل على انفراد لانتخاب التركيز الأمثل للمركب الذي قورت مع السيطرة السالبة،السيطرة الموجبة ومحموعة المقارنة لفيتامين C، بعد ذلك اجري اختبار التداخل مابين التركيز الأمثل والتاريكلورفان استنادا الى معاملتي التاثير قبل قبعداستعمال الترايكلورفان لمعرفة الآلية التي يعمل بها حامض السينناميك في منع وتقليل الأثر السمي للترايكلورفان للكبد فقد عمل على رفع قيمة إنزيمات وظائف الكبدLFTs(ALT,AST,ALP) و كان الفعل أكثر ايجابية عند استعمال حامض السينناميك النقي بجرعة 30 ملغم/كغم بعد الترايكلورفان وبدرجة اقل عند معاملة الحيوانات بحامض السينناميك النقي قبل الترايكلورفان ولذا يمكن تصنيف فعل هذا المركب كونه يعمل على حماية خلايا الكبد من الضرر الحادوكذلك يعمل على وقاية العضلات الهيكلية والقلبية وانسجةالمشيمة.

الكلمات المفتاحية : حامض السينناميك ، الترايكلورفان ، انزيمات وظائف الكبد ، فئران

Introduction

Trichlorfon is an organophosphate insecticide used to control cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leafminers and leaf-hoppers [1]. It also appliedvegetables, fruits and many other field crops and livestock; ornamental and forestry plantings; in agricultural premises and domestic settings; in greenhouses, and for controlfish parasites in designing aquatic environments [2]. It is also used for treating domestic animals to control internal parasites. Trichlorfon is available as dust, Emulsifiable Concentrate (EC), granular, fly bait, and soluble powder formulations. Trichlorfon is a selective insecticide, meaning that it kills targeted pest, but spares many or most other organisms. Trichlorfon is toxic to target insects through direct applications and by ingestion. In other words, it

works as both contact and stomach poison action [2]. Trichlorfon is one of a family of insecticides referred to as organophosphates**,** which may cause delayed symptoms begin 1 to 4 weeks after acute exposure that may or may not produce immediate symptoms , such as numbness, tingling, weakness or cramping which may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, but some residual impairment will remain [3].

Hepatotoxicity is the general term for liver damage [4].The symptoms of hepatotoxicity can be sign in damage of the liver which reflected in liver enzymes levels in the blood ,which are released in to the blood stream .These enzymes levels can be measured by blood tests, which called Liver Function Tests enzymes(LFTs) that are routinely checked as part of LFTs include: Alanine Transaminase (ALT) also called Serum Glutamic Pyruvate Transaminase (SGPT)or Alanine aminotransferase (ALAT), is an enzyme present in hepatocytes (liver cells).When a cell is damage ,it releases this enzyme into the blood ,where it is measured ALT rises dramatically in acute liver damage, such as viral hepatitis or paracetamol overdose. Elevations are often measured in multiple of the upper limit of normal (ULM)[5]. Aspartate Transaminase (AST) also called Serum Glutamic Oxaloacetic Transaminase (SGOT) or Aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It's raised in acute liver damage, but is also present in red blood cells and cardiac and skeletal muscle and is therefore, not specific to liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. Elevated AST levels are not specific for liver damage but have also been used as a cardiac marker [4, 5].Alkaline Phosphatase (ALP) is an enzyme in the cells lining of the billary ducts of the liver. ALP levels in plasma will raise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver, ALP is also present in bone and placental tissue [6].

Most liver diseases cause only mild symptoms initially but it is vital that these diseases be detected early. Hepatic (liver)disorders involvement in some diseases can be of crucial importance.The testing AST,ALT and ALP are liver function tests (LFTs)is performed by a medical technologist on a patient serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality, some with cellular integrity and some with conditions linked to the billary tract (ALP). Liver plays a central role in transforming and clearingtheir chemicals and is susceptible to their toxicity [7]. Certain medical agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemicals agents, such as those used in laboratories and industries, natural chemicals and herbal remedies can also induced hepatotoxicity chemicals that cause liver injury are called hepatotoxins[8].

The bioaviabilities of Polyphenols in plants such as cinnamic acid in cinnamon bark (with all kinds), grape fruit and other and their ability to prevent tumor formation after entering blood circulation and absorbing by bowel. They work directly as in inhibitors by their effect on protein or control factors which operate in there activation the system repairing cell and also because of motivate immune system and increasing conformation natural killer cells and effect on enzymes which are responsible of process and complete the cell cycle by hyperexpression arrangement [9].The pure cinnamic acid is a white crystalline hydroxyl cinnamic acid, slightly soluble in water, it is a part of the biosynthetic shikimate and phenylpropanoid pathways. It is biosynthesis performed by action of the enzyme phenylalnineaminanielyse (PAL)on phenylalanine [10].The derivates of cinnamic acid such as ferulic acid , cinnamicaldehyde, caffeic acid,chlorogenic acid and others sowed ability to cure some disease such as antioxidant in vitro and prevention of type 2-Diabetes Mellitus and cardio vascular diseases and because the scientific and locals tends to use the natural products specially the graces in medical and nutritional yields that made us to focus our immediately study to evaluated the liver function enzymes effects of pure cinnamic acid in one of the biosystem by usingmice [11].

Materials and Methods

Materials:

Trichlorfon 97.8%.

Cinnamic acid CA 98%.

Phosphate Buffer Solution PBS [12].

Colchicine1mg(tablet 1 mg)1 ml Sterile distilled [13].

Note: The solution used immediately after preparing 2.5 to 3 hours

All chemical of high purity were obtained from local marker.

Animals:

White mice aged 8-12 weeks were used, were obtained from National Center for Drug Control and Research, The mice were placed in plastic cages in groups depending on the experiment conditions, at room temperature 25-32Ċ, then provided with water and fed with compound that manufactured locally.

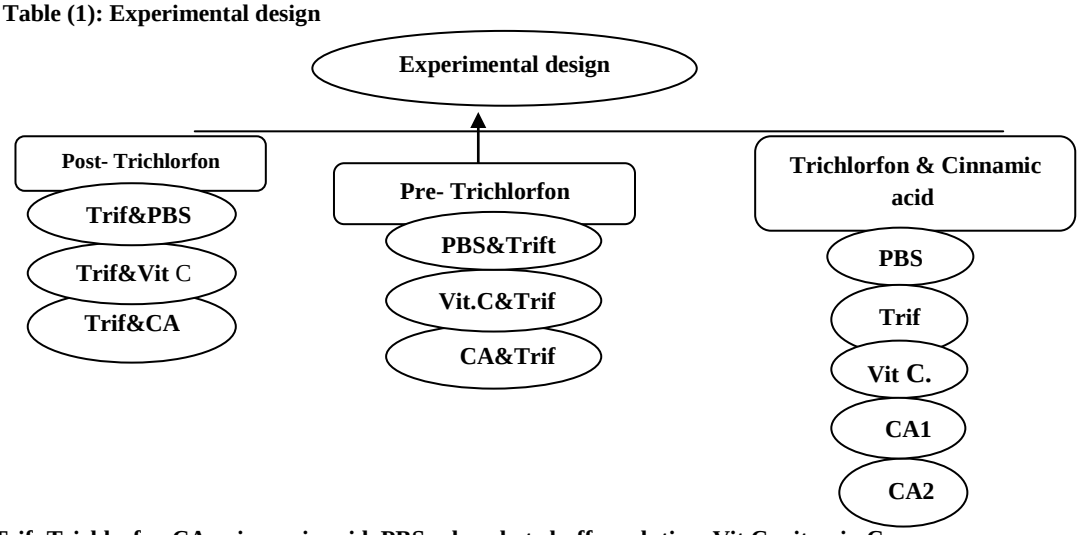
Doses :

Two doses of pure cinnamic acid (Riedel-de Haën company)(CA1=60 ,CA2=30) mg/ Body weight [12], vitamin C(180 mg/ body weight) used comparative groups [14] and Trichlorfon pesticide in (10 mg/ body weight)as a positive control [16] and the PBS as a negative control [12].

Experimental design:

To study the liver function enzymes in laboratory animals ,the gulping was done orally by syringe of 1 ml size supplying with gulping instrument as thin flexible plastic tube to turning shape and soft edge to avoid harming the mouse and inserted to the it's digestive system while trichlorfon solution was injected Intraperitonially [17]. Fifty five mice, weighing 200-250 g were divided into six groups as per the given experimental plan Table (1).

Table (1): Experimental design



***Trif=Trichlorfon,CA= cinnamic acid ,PBS=phosphate buffer solution, Vit C=vitamin C.**

Enzyme assays:

Diagnostic kits from Sigma used to assay ALT, AST and ALP by the following the companying instructions [18].

Statistical analysis:

The program used to compare the effects of treatments in different trails. The least significant difference (LSD) test used to signify a comparison between the means [19].

Results and Discussion

Liver Function Tests for 7 days and trichlorfon.

The effect of perfect dose of cinnamic acid (30mg/body weight) for 7 days treatment in mice showed lack of influence, but there was distinct changes in color and thickness of hair, eyeshape and weight, while cinnamic acid dose (60mg/body weight) showed decrease in weight and changes in hair color to light yellow with less thickness and absence of hair in some parts of the body, beside that changes in the eyes shape and lose their brightness, other side, showed increasing in LFTswhen compared with the negative, positive treatment and vitamin C because high dose of vitamins and minerals can be toxic [20].The dose (30mg/body weight) of cinnamic acid showed remarkable results increase inweight,increase vitality , highly increasing appetite, increasing hair thickness getting more whitish than the normal and the eyes were bright but showed changes in shape.

Trichlorfon was causeddecrease vitality, losing theback hair, legs, and shoulders. More than 20%of the dosed radioactivity is expired as CO_2 [21].This indicates extensive and rapid metabolism in mice .Metabolites were identified in plasma,urine and bile [22] using HPLC,MS,NMR GC-MS and LC-MS.They are rearrangement to form dichlorvos,that is further metabolized to dichloroacetaldehyde and

then dichloroacetic acid;replacement of Cl by H to form the $CHCl_2$ group; formation of a glucuronide at the OH group; demethylation of OCH_3 group to an OH; the two diastereoisomers, M1 and M2 are converted to M3 by removal of HCl; M3is further metabolized to either M13 or dichloroacetic acid [23].The metabolites except dichlorvos and parent drug, are assigned M numbers similar to those used the sponsor. The diastereoisomers, M1 and M2 formed from trichlorfon and M9 and M12 from M10 have been identified.Thebiotransformation to M1 and M2 and M3 was a major pathway, however, more M1 isomer than M2 was found in plasma and urine indicating steroselective glucuronidation.MetabolitesM1, M2 and M3 were the most abundant metabolites.The main trichlorfon biotransformations are shown in figure (1).

Figure (2) expressed that the position treatment with Trichlorfon related to increased value of AST,ALT and ALP enzyme in the serum 45.03,73.21,390.12U/L with significant $p \leq 0.05$ comparing with the negative treatment 35.85,63.62,148.35U/L, while comparative group showed high value in enzyme level 27.24,57.3,272.6U/L and increasing with different significant when compared with both the negative and positive treatment. The group gulped with cinnamic acid of dose 30mg/body weight showed that the value of AST,ALT and ALP were reached to 31.25,59.01,211.54U/L in comparison both negative and positive treatment and no significant with the comparative group $p \leq 0.05$, but during gulping with the dose 60mg/body weight) of cinnamic acid showed significant when compared with the positive treatment and high significant 40.05 ;68.23 ;364.25U/L when compare with both cinnamic acid (60mg/body weight) and also with the comparative group, there is no significant in comparison with the negative treatment group.

The result indicated that 30 mg/body weight was the best dose when gulping for seven days, which showed in the result of AST,ALT and ALP enzymes and referred to caused heart attack, infectious mononucleosis, liver disease inflammation(hepatitis) when the level of this enzyme was increasing [12].

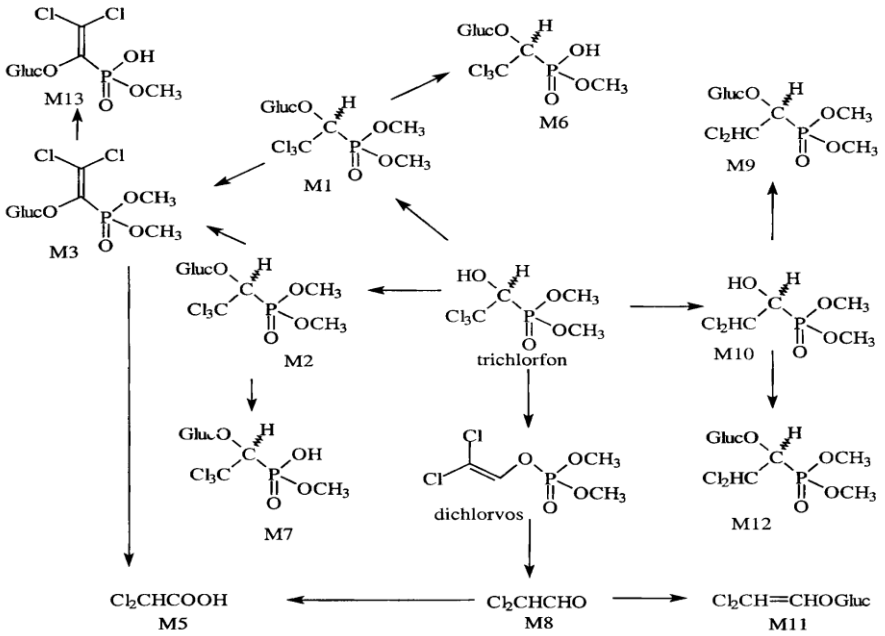


Fig (1):Trichlorfon metabolites

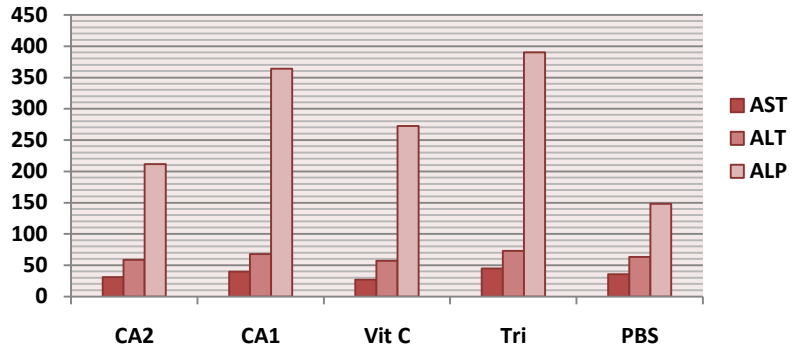


Fig (2): Liver Function Tests for 7 days and trichlorfon
***PBS:Phosphate Buffer Solution;Tri: Trichlorfon ;Vit.C :Vitamin C. ; CA1:cinnamic acid dose (30 mg⁄body weight) ;CA2 :cinnamic acid dose(60 mg⁄ body weight).**

Liver Function Enzymes and the interaction between Trichlorfon and the pure cinnamic acid dose 30 mg /body weight.

After make sure from no hepatotoxicity effects to perfect cinnamic acid concentration which depended in this study, the interaction between the pure cinnamic acid and trichlorfon which caused toxicity and mutation influences because it prevent the cell division by damaging the DNA itself and the interaction contain giving the pure cinnamic acid with dose of 30 mg /body weight after the mutation factor.

Figure (3) showed that treating with Vit. C after Trichlorfon increased the rate of AST,ALT and ALP enzymes concentration in the serum of mice 35.01;59.81;478.89U/L incomparison with control 28.05;52.24;465.32U/L. On the other hand gulping with cinnamic acid with dose of 30mg/body weight after trichlorfon was showed no significant 32.82;61.23;236.74 U/L in comparison with the Vit. C with p value $p \leq 0.05$

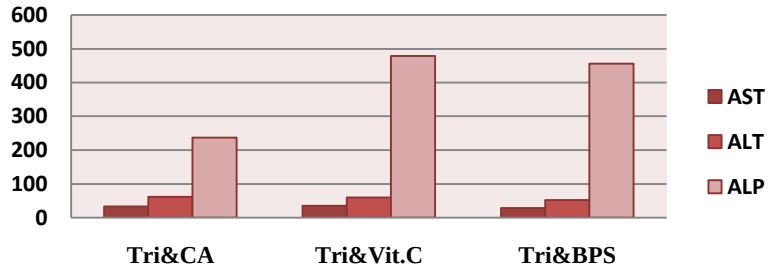


Fig (3): Liver Function Enzymes and the interaction between Trichlorfon and the pure cinnamic acid dose 30 mg /body weight.
****PBS: Phosphate Buffer Solution; Tri: Trichlorfon; Vit.C: Vitamin C.; CA: cinnamic acid dose (30 mg ⁄body weight).**

The antioxidant activity of cinnamic acid and its derivatives is well known in literature [13, 24].Among of cinnamic acid derivatives, caffeic acid has been reported to exhibit good antioxidant and free radical scavenging activities [25].The antioxidant activities of different cinnamic acid derivatives have been reported earlier, and p-coumeric acid, bearing a structural similarity to cinnamic acid, possesses moderate antioxidant activity [26]. The antioxidant activity is of cinnamic acid has ability to act as antioxidant as in the following ways:

1. Phenolic hydroxyl groups are good hydrogen donors [27]. Hydrogen donating antioxidants can react with react oxygen and reactive nitrogen species and breaks the cycle of generation of new radicals [28].
2. 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 [28, 29].

3. 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 [29].
4. Antioxidant capacity of phenolic compounds is also attributed to ability chelate metal ions involved in production of free radicals [28]. 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 [29].
5. 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 [28].

Conclusion

1. Trichlorfon pesticide has oxidation effects on mice liver function enzymes (AST, ALT, and ALP).
2. Cinnamic acid dose 30mg ⁄ body weight has no oxidation effects on mice liver function enzymes (AST, ALT and ALP).
3. The toxic which reflected from trichlorfon eliminated and protected in post- trichlorfon clearly more than pre-trichlorfon, by using of cinnamic acid.
4. Cinnamic acid can considered as Desmutagens at first degree and Bioantimutagens at second degree.

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