

Cinnamic acid activity towards Trichlorfon pesticide and liver function enzymes in rat.

Nibal ,Kh.,Mousa¹;Saad,H.,Khudiar¹,Eman, A.Muhsin¹,Shahad,Sh.,Sabbar¹;
Ishrak, Ab. Ahmed².

1. Ministry of Science and Technology, Baghdad, Iraq.

2. The National Center for Drug Control and Research ,Ministry of Health

Correspondence should be sent to: eman200083@yahoo.co.uk

Abstract :

The study was carried out to determine hepatotoxicity and hepatoprotective effects for cinnamic acid in comparison with vitamin C against the mutagenic pesticide influence of trichlorfon ,which is a chemical compound that damage hepatic cells and has mutagenic effects .The effect was studied in mammalian system *invitro* depended on evaluating the enzymatic activity of three hepatic enzymes: Alanine Transaminase(ALT),Aspartate Transaminase (AST),Alkaline Phosphate (ALP).Two concentration of pure cinnamic acid (60 ,30) mg/kg were evaluated to chose the suitable concentration which remembered the negative control. In order to used in the interaction experiments, included two types to 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 instead it showed highly performance in preventing or reducing the hepatotoxicity of trichlorfon.Cinnamic acid increased the ALT,AST and ALP enzymes with nutral levels especially in dose 30 mg/kg .The positive effect was higher were pure cinnamic acid was 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 at first degree and responsible at the second degree as a cardiac ,skeletal muscle and placental tissue protective.

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

Introduction

Trichlorfon is an organophosphate insecticide used to control cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leafminers and leaf-hoppers [1]. 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 [2]. It is also used for treating domestic animals for control of internal parasites[3]. Trichlorfon is available in dust, emulsifiable concentrate, granular, fly bait, and soluble powder formulations [4]. 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 [2]. Trichlorfon is one of a family of insecticides referred to as organophosphates. Some organophosphates, including trichlorfon, may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure which may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness and cramping 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 [5].

Hepatotoxicity is a general term for liver damage [6]. The symptoms of hepatotoxicity can be sign in damage of the liver which reflected in liver enzyme levels in the blood, when the liver is damaged, there enzymes are released in to the blood stream, where the levels can be measured by blood tests, these are called Liver Function Tests enzymes (LFTs) [7] 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) [8,9]. 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. Its 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 and AST has also been used as a cardiac marker [4,5]. Alkaline Phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma will rise with large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. ALP is also present in bone and placental tissue [10].

Most liver diseases cause only mild symptoms initially but it is vital that these diseases be detected early. Hepatic (liver) 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 biliary tract (ALP) [11]. Liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents [12]. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ [13]. 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 [14]. The bioavailabilities of Polyphenols in plants such as cinnamic acid in cinnamon bark (with all kinds), grape fruit and other and their ability to inhibition and prevent tumor formation after entering blood circulation and absorbing by bowel. They work directly inhibitor by effect on protein or control factors which operate in active the system repairing cell [15] and also because of motivate immune system and increasing conformation natural killer cells and effect in the enzymes which responsible of process and complete the cell cycle by hyperexpression arrangement [16]. The pure cinnamic acid is a white crystalline hydroxyl cinnamic acid, slightly soluble in water, it's a part of the biosynthetic shikimate and phenylpropanoid pathways. It is biosynthesis performed by action of the enzyme phenylalanine aminotransferase (PAL) on phenylalanine [17]. The derivatives of cinnamic acid such as ferulic acid, cinnamaldehyde, caffeic acid, chlorogenic acid and others showed ability to cure some disease [18] such as antioxidant in vitro and prevention of type 2-Diabetes Mellitus and cardiovascular diseases and because the scientific and locals tends to use the natural products specially the grapes in medical and nutrition 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, rats [19].

Materials and Methods

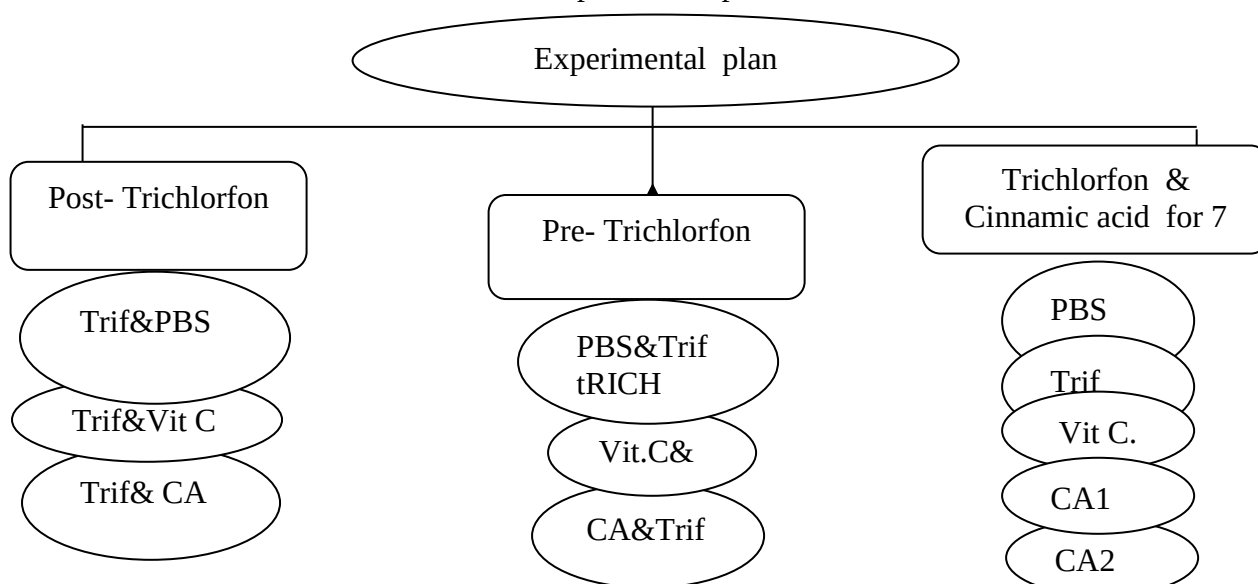
Materials : Trichlorfon (97.8%) and Cinnamic acid (CA) (Riedel –de Haën, Germany ,98%) were purchased locally and all other chemicals used in this study were of high purity and purchased locally. Phosphate Buffer Solution(PBS) [20]. Colchicine 1mg (one tablet) and sterile distilled water 1ml .The solution was used immediately after preparing 2.5 to 3 hours.[21].

Animals : White rats with age rang (8-12) weeks were used, which obtained from National Center for Drug Control and Research ,The rat put in plastic cages in groups depend on the experiment condition, at room temperature (25-32)°C, then gave the water and feeding with compound that manufactured locally.

Doses : Two doses from the pure cinnamic acid (Riedel-de Haën company) which were (CA1=60 ,CA2=30) mg/ Kg[20], vitamin C(180 mg/ kg)as comparative groups[23] and Trichlorfon pesticide in (10 mg/ kg)as a positive control(15) and the PBS as a negative control[20].

Experimental plan : To study the liver function enzymes in laboratory animals ,the gulping was orally by syringe 1 ml size supplying with gulping instrument as thin plastic tube to turning shape and soft edge to avoid harm the rat and inserted to the digestive system of rat ,but trichlorfon solution was injected Intraperitoneal membrane because it lost after (3-12) hours by urine[24], gave the water and integrated animal fed which manufacture locally. Thirty four rats, weighing 200-250 g were divided into six groups as per the given experimental plan [Table \(1\)](#).

Table 1: Experimental plan



*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 accompanying procedures[25]

Statistical analysis : The program was 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[26].

Results and Discussion

Liver Function Tests for 7 days and trichlorfon.

The effect of the perfect concentration of cinnamic acid (30mg / kg) for 7 days treatment in rats were showed lack of influence, other denoted like changes in color and thickness of hair, eyes shape, change in weight, while cinnamic acid concentration (60mg/kg) showed lowing in weight and changes in color of 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 when compared with the negative, positive treatment and vitamin C because high dose of vitamins and minerals can be toxic [27].The concentration (30mg/kg) of cinnamic acid showed excellent results in increasing weight, active in moving, increasing the ability to eat more, increasing hair thickness to be more white than the normal and the eyes were bright with no changes in shape.

Trichlorfon was caused inactive in moving, losing the hair in the back, legs, shoulders and increasing. More than 20% of the dosed radioactivity is expired as CO_2 [28]. This indicates extensive and rapid metabolism in rats. Metabolites were identified in plasma, urine and bile [29] 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; M3 is further metabolized to either M13 or dichloroacetic acid [30]. 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. The biotransformation to M1 and M2 and M3 was a major pathway, however, more M1 isomer than M2 was found in plasma and urine indicating stereoselective glucuronidation. Metabolites M1, 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 ; 390.12) U/L with significant ($p \leq 0.05$) comparing with the negative treatment (35.85 ; 63.62 ; 148.35) U/L while comparative group showed high value in enzyme level (27.24 ; 57.3 ; 272.6) U/L and increasing with different significant when compared with both the negative and positive treatment. The gulping with cinnamic acid (30mg/kg) showed that the value reached of AST, ALT and ALP to (31.25 ; 59.01 ; 211.54) U/L when comparison with both negative and positive treatment and no significant with the comparative group ($p \leq 0.05$), but when gulping with the concentrate (60mg/kg) of cinnamic acid showed significant when compared with the positive treatment and high significant (40.05 ; 68.23 ; 364.25) U/L when compare with both cinnamic acid (60 mg/kg) and also with the comparative group, there is no significant in compared with the negative treatment

The result indicated that (30 mg/kg) was the best concentration 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 hepatitis and trauma when the level of this enzyme was increasing [20].

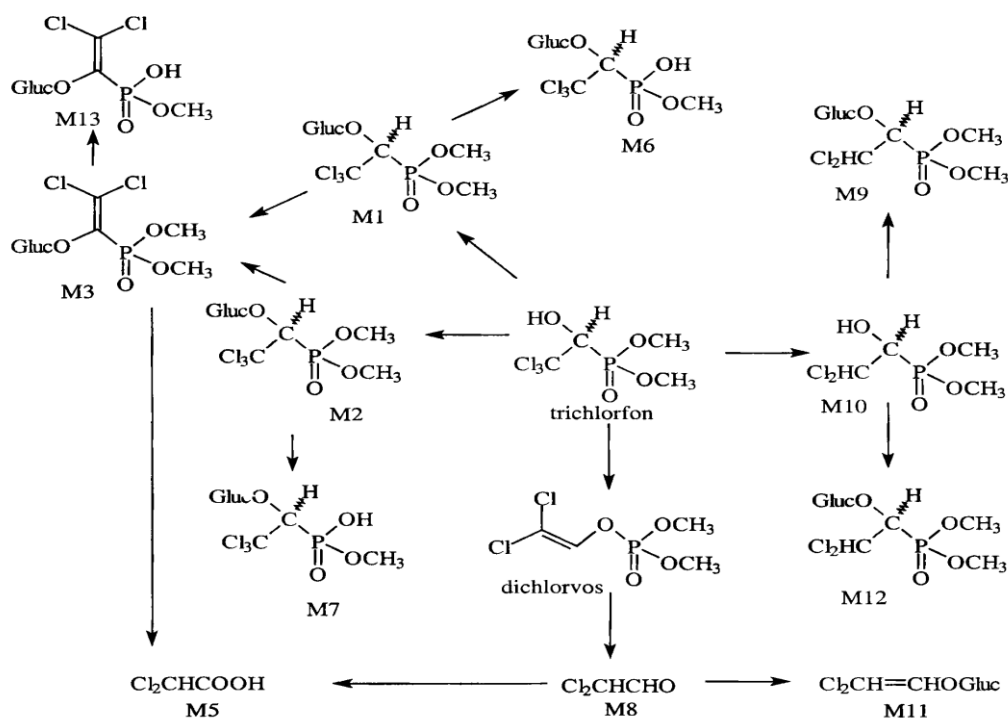
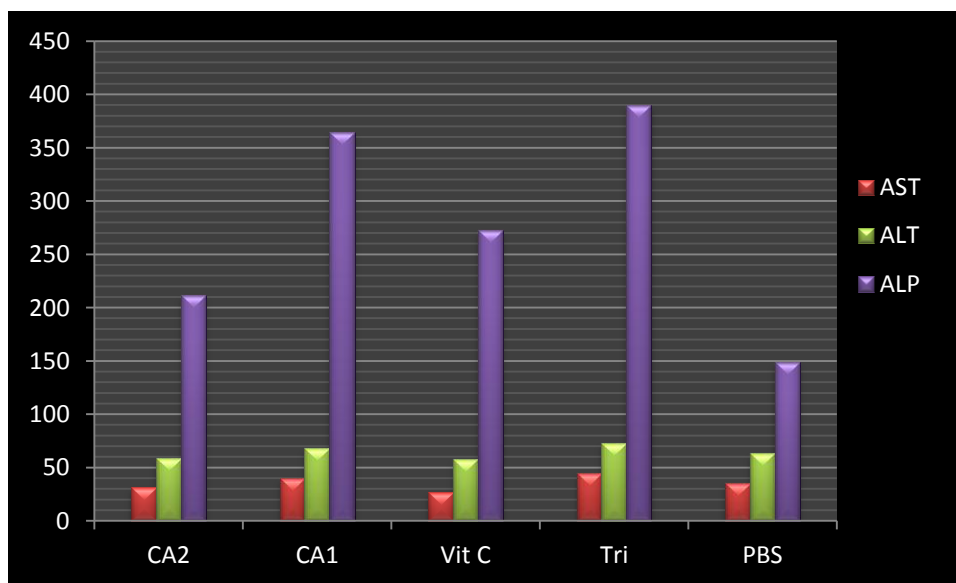


Figure [1].Trichlorfon metabolites



Figure[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/kg) ;CA2 :cinnamic acid dose(60 mg/kg).

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

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

from divided by damaging the DNA itself and the interaction contain giving the pure cinnamic acid with dose 30 mg /kg 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 rats (35.01;59.81;478.89)U/L comparison with control (28.05;52.24;465.32)U/L. When gulping with cinnamic acid dose (30mg/Kg)after trichlorfon was showed no significant (32.82;61.23;236.74) U/L when compared with the Vit. C with p value ($p \leq 0.05$)

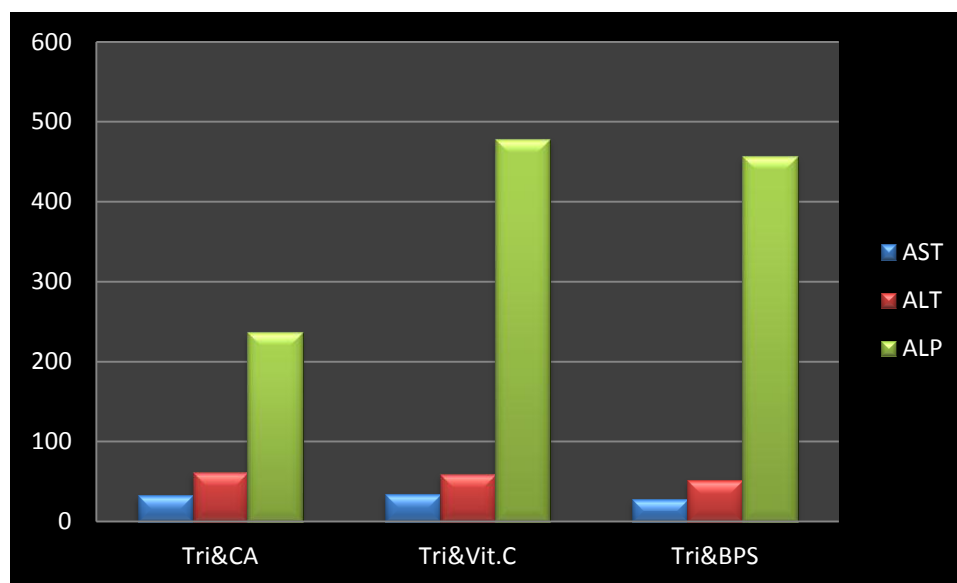


Figure [3]: Liver Function Enzymes and the interaction between Trichlorfon and the pure cinnamic acid dose 30 mg /kg.

**PBS: Phosphate Buffer Solution ;Tri: Trichlorfon ;Vit.C :Vitamin C. ; CA: cinnamic acid dose (30 mg/kg).

The antioxidant activity of cinnamic acid and its derivatives is well known in literature[21,31].Among of cinnamic acid derivatives, caffeic acid has been reported to exhibit good antioxidant and free radical scavenging activities[32].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[33]. The antioxidant activity is of cinnamic acid able to act as antioxidant as in the following ways:

1. Phenolic hydroxyl groups are a good hydrogen donors [34]. Hydrogen donating antioxidants can react with react oxygen and reactive nitrogen species [35,36] and breaks the cycle of generation of new radicals [37].
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 [37,38].
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 [35]. Formation of these long-lived free radicals is able to modify radical-mediated oxidation processes [39].
4. Antioxidant capacity of phenolic compounds is also attributed to ability chelate metal ions involved in production of free radicals [38]. 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 [39].
 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 [36,37].

Conclusion

1. Trichlorfon pesticide has oxidation effects on rat liver function enzymes (AST, ALT, ALP)
2. Cinnamic acid dose (30mg / kg) has no oxidation effects on rat liver function enzymes (AST, ALP, ALP).
3. The toxic which reflected from trichlorfon removed and protection in post-trichlorfon clearly more than pre-trichlorfon.
4. Cinnamic acid can considered as Desmutagenes at first degree and Bioantimutagenes at second degree.

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فعالية حامض السيناميك تجاه مبيد الترايكلوروفان وانزيمات وظائف الكبد في الجرذ.

- نبال خليل موسى¹، سعد حسين خضير¹، ايمان عباس محسن¹، شهد شكري صبار¹، إشراق عبد الأمير علي²
1. وزارة العلوم والتكنولوجيا-بغداد-العراق 2.جامعة برمنكهام ،لندن /المملكة المتحدة
3.المركز الوطني للرقابة والبحوث الدوائية،وزارة الصحة-بغداد-العراق.

الخلاصة

أجريت الدراسة للكشف عن التأثير السمي للكبد لحامض السيناميك النقي cinnamic acid ومقارنته بفيتامين C تجاه مبيد الترايكلوروفان Trichlorfon والذي يعد مركب كيميائي يسبب تلف خلايا الكبد وباستخدام نظام اللبائن في *invitro* وباعتماد على تقييم لثلاث من إنزيمات وظائف الكبد:

Alanine Transaminase(ALT),Aspartate Transaminase (AST),Alkaline Phosphate (ALP).

استخدم تركيزين لحامض السيناميك النقي(30،60) ملغم /كغم وكل على انفراد لانتخاب التركيز الأمثل للمركب والذي أعطى نتائج أفضل من الحالة الطبيعية السيطرة السالبة،بعد ذلك اجري التداخل مابين التركيز



الأمثل والتاريكلورفان وبشكل معاملتان قبل وبعد لمعرفة الآلية التي يعمل بها حامض السيناميك في منع و تقليل الأثر السمي للكبد للترايكلورفان فقد عمل على رفع قيمة إنزيمات وظائف الكبد ALT,AST,ALP)LFTs (وقد كان الفعل الأكثر ايجابية عند استعمال حامض السيناميك النقي بجرعة 30 ملغم/كغم بعد السايكلوفوسفومايد وبدرجة اقل عند معاملة الحيوانات بحامض السيناميك النقي قبل التاريكلورفان وبالتالي يمكن تصنيف فعل هذا المركب في نظام اللبائن كونه علاج بالدرجة الأولى لحماية خلايا الكبد من الضرر الحاد وبالدرجة الثانية وقائياً للعضلات الهيكلية والقلبية وخلايا المشيمة.