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Antioxidant activity of Cinnamic acid against Trichlorfon in mice

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Abstract: The study was carried out to determine the toxic ,oxidant and antioxidant effects of cinnamic acid in comparison with Vitamin C towards the reduce effect of trichlorfon pesticide ,which is a chemical compound that damage hepatic cells and has mutagenic effects. The effect was studied in mice and the bioassay system depended on evaluating the enzymatic activity of two antioxidant enzymes: Catalase and Glutathione Reductase .Two concentrations of pure cinnamic acid 60 and 30 mg/ body weight were evaluated to choose the suitable concentration . In order to use it in the interaction experiments included two types of treatment pre-Trichlorfon and post – Trichlorfon to determine the mechanisms of pure cinnamic acid which showed no toxic and oxidant effects in biological system and it showed highly performance in preventing and reducing the oxidant stress influences of trichlorfon. It was increased the catalase and glutathione reductase antioxidant activity especially in dose 30mg/ body weight. The positive effect was higher when pure cinnamic acid was used as post- trichlorfon treatment and to less extent in pre –Trichlorfon treatment. The cinnamic acid can be considered as an antioxidant compound and Desmutagen in the first degree and Bioantimutagen in the second degree

Key words: Cinnamic acid, Trichlorfon, Catalase, Glutathione Reductase, Mice.

فعالية تضاد الاكسدة لحامض السينامیك على بقايا مبيد تريكلوروفان في الفئران المختبرية

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الخلاصة: أجريت الدراسة للكشف عن التأثير السمي المؤكسد والمضاد للأكسدة لحامض السينامیك النقي cinnamic acid ومقارنته بفيتامين C تجاه بقايا المبيد ترايكلورفون Trichlorfon والذي يعد مركبا" كيمائيا" يسبب تلف خلايا الكبد ويمتلك تأثيرا سميا" مؤكسدا"، وباستخدام نظام اللبائن في الفئران المختبرية وبالاعتماد على تقييم الفعالية الإنزيمية للمضادين للأكسدة: الكاتليز وكلوتاثايون المختزل لمجانس خلايا الكبد.استخدم تركيزين لحامض السينامیك النقي(30 ،60)ملغم /وزن الحيوان، وكل على انفراد لاختبار فعلها المؤكسد والمضاد للاكسدة وتم انتخاب التركيز الامثل للمركب والذي اعطى نتائج افضل من الحالة الطبيعية السيطرة السالبة. اجري التداخل مابين التركيز الامثل والمؤكسد للترايكلورفون Trichlorfon وبشكل معاملتين قبل وبعد العامل المؤكسد لمعرفة الالية التي يعمل بها هذا المركب في منع او تقليل الاثر التاكسدي للمبيد فقد عمل على زيادة فعالية انزيمي الكاتاليز والكلوتاثايون المختزل المضادين للاكسدة وقد

كان الفعل الاكثر ايجابية عند استعمال حامض السينامیك النقي بجرعة 30 ملغم /كغم بعد العامل المؤكسد وبدرجة اقل عند معاملة الحيوانات بحامض االسينامیك النقي قبل العامل المؤكسد و يمكن تصنيف فعل هذا المركب في نظام اللبائن كونه مضاد للاكسدة ومثبط مباشر Desmutagens بالدرجة الاولى ومثبط حيوية Bioantimutagens بالدرجة الثانية.

Introduction

Oxidative stress is responsible for various pathological conditions such as cancer, cardiovascular diseases, asthma , arthritis, inflammation, neurodegenerative disorders and dementia (1). Pesticides and insecticides , persistent organic-pollutants that cause oxidative stress in animals, including humans.Trichlorfon is an organo –phosphate insecticide used to control cockroaches, crickets, silverfish, bedbugs, fleas, cattle grubs, flies, ticks, leaf-miners, and leaf-hoppers (2) as well as treating domestic animals for control of internal parasites (3). Moderate to severe symptoms of trichlorfon toxicity have been reported in farm and factory workers, military personnel-and exterminators (4).

The common human symptoms of trichlorfon toxicity include nausea, diarrhea, vomiting, abdominal cramps, headache, dizziness, eye pain, blurred vision, tears, sweating, and confusion. Severe poisoning may affect the activity of the central nervous system leading to in-coordination, slurred speech, loss of reflexes, involuntary muscle contraction and eventually paralysis of the respiratory muscles and other extremities (5). Endogenous antioxidants are the essential defense weapons of the cell against free radicals and oxidative damage. However, chronic exposure to pesticides has led to the induction of oxidative stress beyond the protecting efficiency of the endogenous antioxidants. Exogenous antioxidants from natural plant sources have been widely used to counteract pesticide-induced oxidative stress(6). Phenolic acids are widespread in the plant kingdom and are known to possess a number of biological activities (7). Because the Scientifics and local tends to use the natural products specially the graces in medical and nutrition yield that made us to focus our immediately study to evaluated the hepatoprotective effect of pure cinnamic acid in one of the biosystem.

Materials and Methods

Phosphate Buffer Solution (PBS) (9). Beta-Nicotinamide Adenine Dinucleotide Phosphate Sodium Salt(NADPH+H)(1Mm) (9).Hydrogen peroxide (H_2O_2) prepared from mixing 0.112 ml from H_2O_2 (30%) with 100ml from PBS (10) . Bovine Serum Albumin (BSA)1% prepared by dissolving 1g BSA in 100 ml sterile distiller water (10).Colchicine Solution prepared by1mg dissolving of colchicines tablet in one milliliter from sterile distiller water and inject mouse with 0.25 ml from colchicines solution in intraperitoneal membrane before anatomy(2.5-3)hours and it used immediately(11).Biuret solution preparing is to (10).

Doses

Two doses from the pure cinnamic acid were (CA1=60 , CA2=30) mg / body weight, vitamin C(180mg/ body weight)as comparative groups(12) and

Trichlorfon pesticide in(10 mg/body weight)as a positive control(13) and the PBS as a negative control(9).

Experimental plan

To study the oxidant effect and the antioxidant in laboratory animals: The Cinnamic acid was gulping orally by syringe 1 ml size .The Trichlorfon solution was injected Intraperitonially because it lost after (3-12) hours by urine (13,14).Fifty -five mice weighing 20-25 g were divided into three groups as in experimental plan, Figure (1) :

1st groups: we have taken perfect cinnamic acid concentration consist of (negative control (PBS) and positive control (Trichlorfon) comparative group. vitamin C. and two cinnamic acid concentration (30.60) mg / body weight).

2nd groups: pre(before)-Trichlorfon.

3rd groups: post(after) –Trichlorfon.

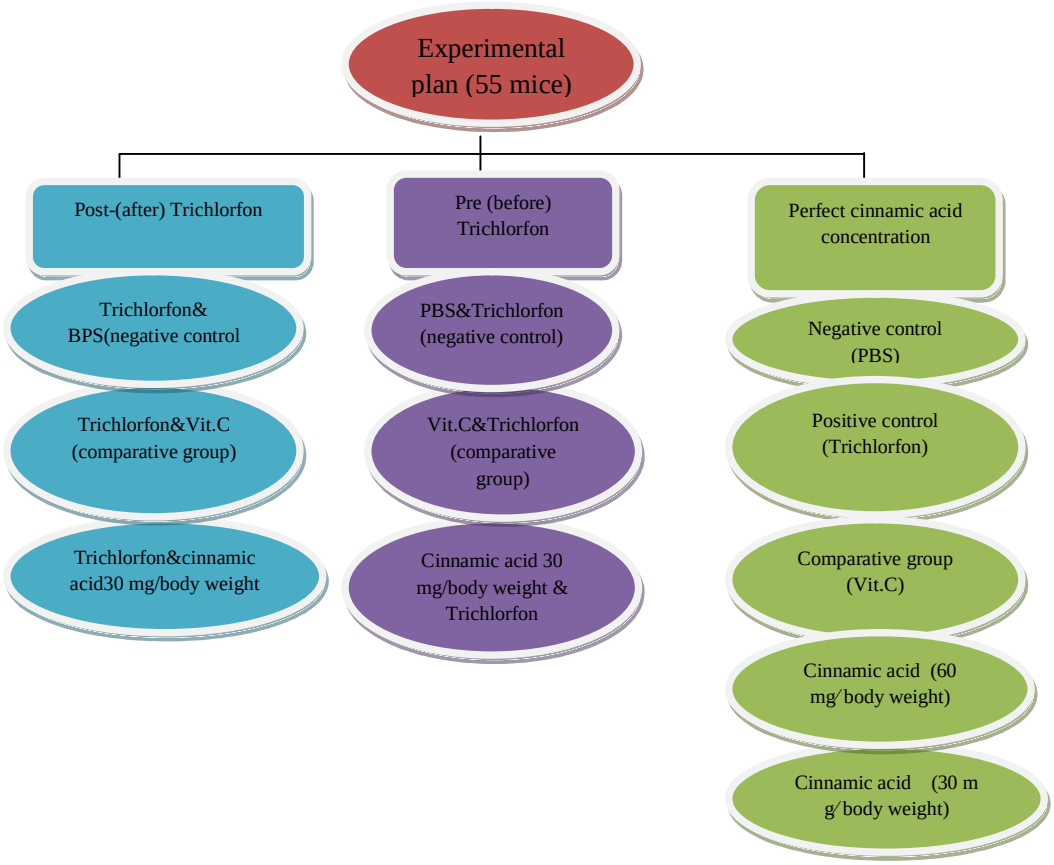

Figure (1): Experimental plan

Preparing of liver mouse serum

Weight 1 g from the liver kept in freez and cut it to very small pieces by sharp knife in 1 ml from PBS and using in the same time the mechanism pressure of hand to crush the liver tissue till be sticky solution then move the rest to the centrifuge with9,000 rpm for 20 minutes .Get the upper layer and let the remainder in the bottom of the test tube avoid the fatty layer above it and store in freezer (-20)Ċ until evaluation (8,10).

Evaluation the Glutathione Reductase

To evaluate the activity of enzyme in volume 1ml,contains interaction mixture: 0.1 ml from BSA 1%. , 0.4 ml from oxidation Glutathione , 0.4 ml from 1mM NADPH+H.,0.1ml from the enzyme extraction then read the interaction mixture in spectrophotometer in 340 nm and recorded the reading every 30 second for half an hour.

The Glutathione Reductase (GR) evaluation based on moving hydrogen of $NADPH_2$ to the oxidation Glutathione then measure the interaction by spectrophotometer: the continuous of interaction led to the reduction of absorbance as a result to change the oxidized GSH to reduced GSH. The unit of enzyme evaluation based on the reduction of the amount of absorbance through one minute under the standard interaction (18, 19).

Evaluation the Catalase

One milliliter of phosphate buffer and 0.4 ml water were added to 0.1 ml of 'BSA', the reaction was started by adding 0.5 ml H_2O_2 ,the mixture was incubated at 37℃ for 1 minute the reaction was stopped by adding 2 ml of dichromate: acetic acid reagent and kept in a boiling water bath for 15 minutes. The mixture was cooled and absorbance was read at 570 nm. (15).

Evaluation the amount of protein

2ml of sterilize distilled water was added to the first tube (blank) then we add 2ml standard solution of protein to the second tube (standard) added 0.2ml from the test (liver mouse extract) and 1.8ml sterilize distilled water to the third tube (test sample) and then added 5ml Biuret solution to the three tubes and mixed well, then put in water bath at 37Ċ for 10min. Cooled and read absorbanent at 50nm and calculated amount of protein(9,10) as:

$$\text{Protein amount (gm / 100ml)} = \text{test absorbance} \times \frac{SCP}{SAP}$$

SCP=Standard concentrate of protein

SAP=Standard absorbance of protein

Statistical analysis

The statistical analysis is done to get the means ±SE and test the different significant among the means by using Duncan test (20) then differences among the means in interaction experiments were compared between the Vit. C, cinnamic extract and the trichlorfon by using T- test (21).

Results and Discussion

Antioxidant enzymes results for 7 days

Catalase enzyme (CAT for 7 days)

The results in figure (1) showed lowing in activity of catalase when treated with trichlorfon, the treatment with comparative group showed that there was statistically significant of specific activity comparison with negative and positive treatment with p value($p \leq 0.05$).Among the means-in

interaction experiments were compared between the Vit. C, cinnamic extract and the trichlorfon by using T- test (21).

The cinnamic acid (60mg/body weight) showed significantly in comparative with positive treatment and comparative group while there were no significant with negative treatment. So the cinnamic acid concentration (30mg/body weight) showed no significant comparison with comparative group.

Glutathione Reductase(GR for7 days)

The positive treatment (Trichlorfon) led to low value activity with significant for GR The comparative group gave significant when comparative with negative and positive treatment. Results in figure (2) and table(1)of gulping mice with cinnamic acid concentration(CA1=60mg/body weight) showed significant in comparing with other treatment, while gulping the mice with concentration (CA2=30mg/body weight) of cinnamic acid showed elevating in significantly and no significant when compared with the comparative group ($p \leq 0.05$).

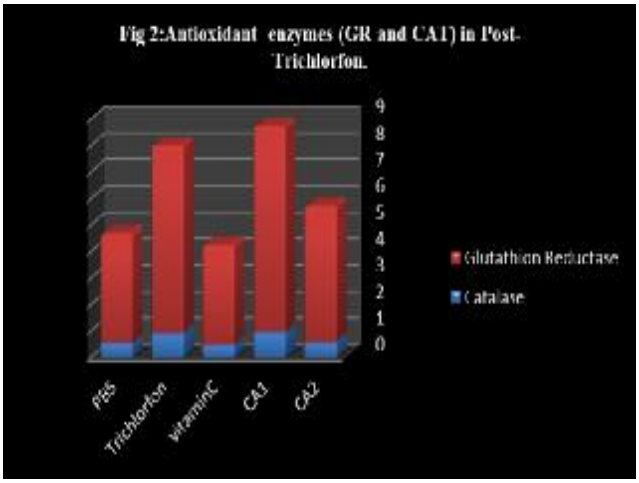

Figure (2): Antioxidant enzymes (GR and CAT) in Post Trichlorfon

Table (1) :Antioxidant activity of GR and CAT in mice

Treatment	Negative control PBS	Positive control Trichlorofon	Vitamine C	Cinnamic acid (60mg/body weight)	Cinnamic acid(30 mg /body weight)
Mean ±SE (U/ mg protein)					
Catalase	0.53±0.13	0.91±0.3	0.16±0.92	0.956±0.82	0.594±0.21
Glutathione reductase	4.132±0.24	7.09±0.05	3.75±0.42	7.77±0.64	5.126±0.29

Antioxidant and activity of GR and Catalase in mice treated with Trichlorfon comparative with positive control

Catalase enzyme (CAT)

The treatment in figure (3) showed that Vit. C after trichlorfon increasing activity in comparison with control treatment.

When gulping with concentration (30mg /body weight) of cinnamic acid after trichlorfon showed increasing in specific activity when compared with both control and Vit. C treatment with p value ($p \leq 0.05$).

Glutathione reductase enzyme (GR)

The treatment in figure (3) and table (2) showed that treating with Vit. C after trichlorfon increasing in specific activity of glutathione reductase when comparison with control treatment. When gulping with (30 mg/body weight) of cinnamic acid after Trichlorfon treatment showed increasing in specific activity when compared with both control and Vit.C treatment with p value ($p \leq 0.05$).

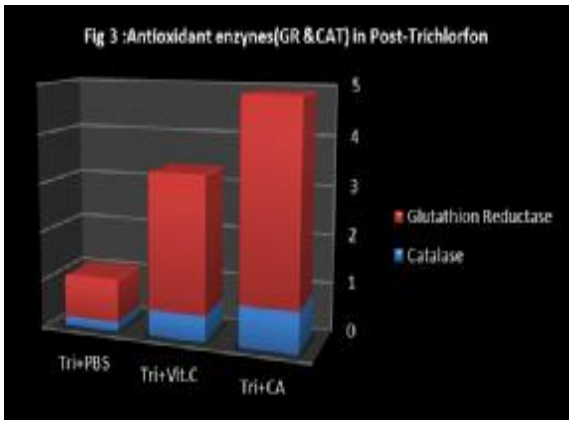

Figure (3): Antioxidant enzymes (GR and CAT) in Post Trichlorfon

Table (2) :Antioxidant enzymes (GR&CAT)in post-Trichlorfon

Treatment / Test	Trichlorfon and Phosphate Buffer solution	Trichlorfon and vitamin C.	Trichlorfon and Cinnamic acid (30 mg/body weight)
Mean ±SE (U/ mg protein)			
Catalase	0.193±0.025	0.534 ± 0.034	0.846 ± 0.003
Glutathione Reductase	0.803 ± 0.032	2.905 ± 0.081	5.085 ± 0.085

Enzymatic antioxidants

Mammalian cells are equipped with antioxidant enzymes to minimize the cellular damage resulting from intracellular ROS (reaction oxygen species) or pesticide-induced oxidative stress (22). Catalase and Glutathione reductase work in a coherent manner to inactivate the ROS generated and Catalase is responsible for the breakdown of H_2O_2 to water and oxygen, protecting the cell from the damaging action of H_2O_2 and the hydroxyl radical. Glutathione reductase catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxides. Organophosphates dichlorvos (trichlorfon) was reported to decrease the activity of these enzymes of mice blood (23) and mice brain (24,25).

Role of cinnamic acid

The antioxidant activity of cinnamic acid and its derivatives are well known in literature (8,29). Among of cinnamic acid derivatives caffeic acid has been reported to exhibit good antioxidant and free radical scavenging activities (29). The antioxidant activities of different cinnamic acid derivatives have been reported earlier, and p-coumeric acid bearing a structural similarity to CA possesses moderate antioxidant activity (29). The antioxidant activity of cinnamic acid able to act as antioxidant as in the following ways: Phenolic hydroxyl groups are good hydrogen donors (30). Hydrogen donating antioxidants can react with reactive oxygen and reactive nitrogen species (31,32) and breaks the cycle of generation of new radicals (33). 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 (33, 34). 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 (31). Formation of these long-lived free radicals is able to modify radical-mediated oxidation processes (33) .

Antioxidant capacity of phenolic compounds is also attributed to ability chelate metal ions involved in production of free radicals (34). However, phenolic compounds can acts as pro-oxidants by chelating metals in manner that maintains or increas 56 catalytic activity or by reducing metals, thus increasing their ability to form free radicals (34) . 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 (33, 34).

Conclusion

-Cinnamic acid dose (30mg⁄body weight) has no oxidation effects on mice liver antioxidant enzymes (GR,CAT).

-The toxic effect which reflected from trichlorfon removed and protection in post- Trichlorfon clearly more than pre-Trichlorfon.

-Because Post-trichlorfon treatment was the best results which reflect cinnamic acid dose(30 mg / body weight) Desmutagenes at first degree due to cinnamic acid ability to stop mutagen toxicity before it damage genetic material DNA and mean

cinnamic acid work outside of cell and called extracellular.

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