# THERAPEUTIC EFFICACY OF ATROPINE AGAINST CHOLINESTERASE INHIBITORS TOXICITY IN CHICKS STRESSED WITH HYDROGEN PEROXIDE

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## ABSTRACT

The aim of this study was to evaluate therapeutic efficacy of atropine in case of cholinesterase (ChE) inhibitors toxicity in chicks subjected to oxidative stress (OS) with hydrogen peroxide ($H_2O_2$). $H_2O_2$ at 0.5 % in drinking water induced OS at age of 7-14 days in the chicks when given each day for 14 consecutive days. There was no change in the acute median lethal dose ($LD_{50}$) for diazinon (7.9 mg/kg, orally) for both $H_2O$ (control) and $H_2O_2$ groups. The therapeutic efficacy of atropine was decreased (265 %) when measuring the median effective dose ($ED_{50}$) for treating the diazinon toxicity in $H_2O$ group (2.6 mg/kg, i.m.) and in the $H_2O_2$ group (9.5 mg/kg, i.m.). The signs of acute diazinon toxicity were found to increase in $H_2O_2$ groups when compared to $H_2O$ group and there is slight decrease in plasma and whole brain ChE activities in $H_2O_2$ group when when compared to $H_2O$ group. The data revealed a decline in atropine efficacy for

treatment diazinon toxicity in chicks that suffered from OS status and it is recommended here to increase the dose of atropine in this case.

## INTRODUCTION

Atropine is the drug of choice which is used as an antidote for the treatment of cholinesterase (ChE) inhibitors toxicity such as organophosphates and carbamates in human and animals by blocking the muscarinic receptors, thereby diminishing the excess acetylcholine from binding to it (1-3). Diazinon is an organophosphate insecticides that induces toxicity through inhibiting acetylcholinesterase in living tissues (2,3). Studies have recommended using the powerful oxidant agent hydrogen peroxide ($H_2O_2$) for induction of OS in laboratory animals such rats and chicks (4-7). It was found that OS modulates the pharmacological response to drugs in chicks and if $H_2O_2$ was given at 0.5 % in drinking water, it induces OS in 7-14 day-old chicks when given fresh each day (5-7). $H_2O_2$ induces OS through its capacity to increase the reactive oxygen species (ROS) and free radicals formation in the tissues (8,9). The aim of the present study was to evaluate the therapeutic efficacy of atropine in case of ChE inhibitors (diazinon as a model) toxicity in chicks suffered from OS induced by $H_2O_2$ since no previous studies dealt with such a life threatening situation.

## MATERIALS AND METHODS

**Experimental animals and induction of OS**

Day-old broiler chicks of both sexes (body weight 45-125 g) were used. They were kept in animal house at a temperature of 32–35 °C with constant lighting, and wood shavings as floor litter. The chicks had free access to drinking water and feed along the

experiment. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul reviewed and approved the protocol of this study. To induce OS, day-old chicks were either provided with tap water ($H_2O$-control group) or with fresh $H_2O_2$ (Thomas Baker Chemical Ltd., U.K.) in tap water as 0.5% v/v each day for two weeks in order to produce an OS as reported in previous studies (4,5,7).

**Determination of acute median lethal dose ($LD_{50}$) of diazinon in $H_2O$ (control) and $H_2O_2$ (stressed) groups**

The acute $LD_{50}$ of diazinon (60%, VAPCO, Jordan) was measured for both the control and stressed groups according to the up-and-down method described before (10). The initial dose of diazinon was 7 mg/kg, orally for both groups. The onset and signs of acute toxicity of diazinon in the chicks during 24 hours were also recorded in this experiment.

**Measuring the median effective dose ($ED_{50}$) of atropine in the control and stressed groups for alteration of acute diazinon toxicity**

After determination of the acute $LD_{50}$ value of diazinon (7.9 mg/kg, orally), the dose of diazinon (10 mg/kg, orally) was chosen that causes acute toxicity leading to lethality in the chicks. The $ED_{50}$ of atropine (1%, Alsharq for Veterinary Drugs, Syria) was determined for both the control and stressed groups according to up-and-down method (10). The onset and signs of acute toxicity of diazinon during 24 hours were measured to evaluate the differences between these two groups.

**Therapeutic efficacy of atropine against acute diazinon toxicity in control and stressed chicks with $H_2O_2$**

Atropine was injected at 6 mg/kg, i.m. (this dose was chosen according to $ED_{50}$ value of atropine) immediately after diazinon dosing at 10 mg/kg, orally for both the control and stressed chicks (6 chicks/group). The onset of acute diazinon toxicity, toxic signs and toxicity scores were evaluated for both groups.

**In vivo determination of ChE activity in plasma and whole brain of the control and stressed chicks**

Atropine was injected at 6 mg/kg, i.m. immediately after diazinon dosing at 10 mg/kg, orally for both the control and stressed chicks (6 chicks/group). The chicks were euthanized and blood samples and whole brain were collected after 2 hours of atropine and diazinon administration. The ChE activity was determined according to a modified electrometric method described earlier (11-13) for investigating the alteration on the level of ChE. Whole brain was homogenized on an ice bath by using a homogenizer in a pH 8.1 barbital-phosphate buffer solution at 3ml/100 mg wet weight of whole blood (11,12). To measure ChE activity, the reaction mixture composed of 3 ml distilled water, 0.2ml plasma or whole brain homogenate and 3ml of pH 8.1 buffer. The initial pH of the mixture ($pH_1$) was measured by using a pH meter (Hanna, Romania), and then the substrate 7.5 % acetylthiocholine iodide was added to the mixture and incubated at $37^0$C for 30 min. Then the pH of the reaction mixture ($pH_2$) should be measured. The ChE activity (ΔpH/30 min.) was calculated as follows:

ChE activity (ΔpH/30 min.)＝($pH_1$−$pH_2$)−ΔpH of blank (The blank was without the plasma or brain homogenate sample).

**Statistics**

To compare the means of two parametric groups, the unpaired Student's t-test was applied for statistical analysis (14,15). On the other hand, the nonparametric data of the two groups were statistically analyzed by using Fisher exact probability test and Mann-Whitney U-test (15,16). The level of significance was at $p < 0.05$.

## RESULTS

**Acute $LD_{50}$ value of diazinon in $H_2O$ and $H_2O_2$ groups of chicks**

The acute $LD_{50}$ value of diazinon was 7.9 mg/kg, orally for both the control and stressed chicks (Table 1). The onset of acute toxicity was decreased and the signs of toxicity (which were salivation, dyspnea, depression, tremor, recumbency, paralysis, convulsion and death) were exacerbated in the stressed group when compared to the control group as demonstrated in Table 1.

Table 1: Acute $LD_{50}$ value of diazinon in the control and stressed chicks with $H_2O_2$

| Parameters | Results | |
|---|---|---|
| | Control group (Tap water) | Stressed group (0.5 % $H_2O_2$ in water) |
| Acute $LD_{50}$ value | 7.9 mg/kg, orally | 7.9 mg/kg, orally |
| The doses range | 7-10 | 7-10 |
| Initial dose | 10 mg/kg | 10 mg/kg |
| Last dose | 10 mg/kg | 10 mg/kg |
| Increase or decrease in the dose | 3 mg | 3 mg |
| Number of chicks | 5 (XOXOX) | 5 (XOXOX) |
| Onset of acute toxicity | 21-48 min. | 5-11 min. |
| Toxicity signs | Salivation, dyspnea, depression, tremor, recumbency, paralysis, convulsion and death | The same as the control with exacerbation |
| % effect of OS on acute $LD_{50}$ value of diazinon= 0 % | | |

X= death; O= survival

**The $ED_{50}$ value of atropine in the control and stressed groups of chicks**

Table 2 shows that the $ED_{50}$ value of atropine was effective in treating the diazinon toxicity increased by 265 %, which demonstrates decreased effectivness in the $H_2O_2$ (stressed) group (9.5 mg/kg, i.m.) when compared with the $H_2O$ (control) group (2.6 mg/kg, i.m.).

Table 2: $ED_{50}$ of atropine for preventing the acute toxicity of diazinon in control and stressed chicks with $H_2O_2$

| Parameters | Results | |
|---|---|---|
| | Control group (Tap water) | Stressed group (0.5 % $H_2O_2$ in water) |
| $ED_{50}$ value | 2.6 mg/kg, i.m. | 9.5 mg/kg, i.m. |
| The range of the doses | 2-4 | 6-10 |
| Initial dose | 4 mg/kg | 6 mg/kg |
| Last dose | 3 mg/kg | 8 mg/kg |
| Increase or decrease in the dose | 1 mg | 2 mg |
| Number of chicks | 6 (XXOOXX) | 6 (OOXOXO) |
| % effect of OS on $ED_{50}$ value of Atropine= 265 % | | |

X= survival; O= death

Atropine was injected (i.m.) immediately after the diazinon administration at 10 mg/5ml/kg, orally

**Therapeutic efficacy of atropine against acute diazinon toxicity in control and stressed chicks with $H_2O_2$**

The signs of acute diazinon toxicity were found to increase in the stressed groups when compared to control groups of chicks by measuring the onset of acute signs, signs of toxicity and toxicity scores which revealed also a decrease in atropine therapeutic efficacy in the stressed chicks when compared to the control tap water group of chicks (Table 3).

Table 3: Therapeutic efficacy of atropine against acute diazinon toxicity in control and stressed chicks with $H_2O_2$

| Groups | Onset of toxicity (min.)+ | % Toxicity signs | | | | | | Toxicity scores |
|---|---|---|---|---|---|---|---|---|
| | | Salivation | Defecation | Tremor | Dyspnea | Recumbency | Paralysis | |
| Control group (tap water) | 49.67 ± 2.88 | 100 | 50 | 16.67 | 16.67 | 100 | 33.33 | 14 |
| Stressed group (0.5 % $H_2O_2$ in water) | 15.67 ± 2.04* | 100 | 50 | 66.67* | 83.33* | 66.67 | 33.33 | 18 |

+ Data represented Mean ± S.E. for 6 chicks/group

* significantly different from the respective control ($H_2O$ group) at $p < 0.05$

Atropine was injected at 6 mg/5ml/kg, i.m. immediately after diazinon dosing at 10 mg/5ml/kg, orally

**ChE activity in the plasma and whole brain of the control and stressed chicks**

Table 4 describes a slight change in the plasma and whole brain ChE activity with more decreased in enzyme activity for the stressed chicks when compared to the control chicks.

Table 4: ChE activity in the plasma and whole brain in the control and stressed chicks with $H_2O_2$

| Groups | Plasma | | Whole brain | |
|---|---|---|---|---|
| | ChE activity (Delta pH/30 min.) | % inhibition | ChE activity (Delta pH/30 min.) | % inhibition |
| Control group (tap water) | 0.048 ± 0.005 | ---------- | 0.030 ± 0.005 | ---------- |
| Stressed group (0.5 % $H_2O_2$ in water) | 0.035 ± 0.008 | 37 | 0.028 ± 0.009 | 7 |

Data represented Mean ± S.E. for 6 chicks/group

Atropine was injected at 6 mg/5ml/kg, i.m. immediately after diazinon dosing at 10 mg/5ml/kg, orally

Blood samples and whole brain were collected after 2 hours of atropine and diazinon administration

## DISCUSSION

Many cases of poisoning and diseases in animals may be accompanied by stressful conditions that may affect the pharmacological responses and therapeutic efficacy of certain drugs. The goal of this study was to evaluate the therapeutic efficacy of atropine in case of ChE inhibitors (diazinon as a model here) toxicity in chicks suffered from the OS (as a model of stress) induced with a powerful oxidant $H_2O_2$ since

no previous literature dealt with that a life threatening situation. Fresh $H_2O_2$ given each day at 0.5 % in drinking water was found to induce OS in 7-14 day-old chicks old (4,5) which were found by reduced glutathione and elevation of malondialdehyde concentrations in the body tissues of chicks. Atropine is the drug of choice used as an antidote for the treatment of ChE inhibitors (organophosphates and carbamates) toxicity and preventing death in human and animals through blocking the muscarinic receptors (1-3). Diazinon induces toxicity through inhibiting acetylcholinesterase in living tissues (2,3). There was no change in the acute $LD_{50}$ value for diazinon in both the control and stressed groups of chicks and it may be atributed to acute organophosphate toxicity during 2 hours after dosing and death in vulnerable chicks (17). The therapeutic efficacy of atropine at all was decreased when measuring the $ED_{50}$ of atropine for treating the diazinon toxicity and death in the control and stressed chicks and this revealed that atropine was not able to treat and counteract diazinon toxicity at the same time with the presence of a stressful condition (OS induced with $H_2O_2$ as a model) because stressful conditions have synergistic action with toxicity status that exacerbate this condition (18). At the same time, the OS induced with $H_2O_2$ modulates the pharmacological response and efficacy of drugs in animals (4-7). In the same manner, the signs of acute diazinon toxicity were found to increase in stressed chicks with $H_2O_2$ when compared to groups that received tap water. A similar effect was described in previous study (18). There was a slight change in the plasma and whole brain ChE activity in stressed chicks when compared to the control chicks which means that the OS status caused by $H_2O_2$ affect the enzyme level and potentiates the toxicity status (18). The data revealed a decline and modulation in atropine efficacy for treatment of ChE inhibitors (diazinon) toxicity in the

chicks that suffered from OS status induced with $H_2O_2$. We recommend here an increase the dose of atropine in order to improve the treatment in this case.

## الفعالية العلاجية للأتروبين ضد التسمم بمثبطات خميرة الكولين أستراز في الأفراخ المجهدة ببيروكسيد الهيدروجين

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### الخلاصة

تهدف الدراسة الى تقييم الفعالية العلاجية للأتروبين في حالة التسمم الحاد بمثبطات خميرة الكولين أستراز في نموذج أفراخ الدجاج والتي تعاني من الإجهاد التأكسدي المحدث باستخدام بيروكسيد الهيدروجين حيث أنه لا توجد دراسات سابقة أخذت بنظر الاعتبار هذه الحالة. وقد وجد أن إعطاء بيروكسيد الهيدروجين بتركيز 0.5٪ في ماء الشرب يعمل على إحداث الاجهاد التأكسدي في أفراخ الدجاج بعمر 7-14 يوم عندما يعطى بصورة طازجة كل يوم. لم يكن هناك تغيير في الجرعة المميتة الوسطية الحادة للديازينون (7,9 ملغم / كغم، عن طريق الفم) لكل من المجموعة التي أعطيت ماء الشرب وتلك التي اعطيت بيروكسيد الهيدروجين مع ماء الشرب. وعند قياس الجرعة الفعالة الوسطية للأتروبين لكل من المجموعة التي أعطيت ماء الشرب (2,6 ملغم / كغم، في العضل) وتلك التي اعطيت بيروكسيد الهيدروجين مع ماء الشرب (9,5 ملغم / كغم، في العضل) فقد تبين أن هاك انخفاض في الفعالية العلاجية (265٪) لعلاج التسمم الحاد بالديازينون في أفراخ الدجاج. وعند العلاج بالأتروبين فقد لوحظ زيادة العلامات السمية الحادة للديازينون في المجموعة المعاملة ببيروكسيد الهيدروجين بالمقارنة مع المجموعة المعطاة ماء الشرب وفي الوقت نفسه هناك تغييرات طفيفة في نشاط خميرة الكولين أستراز في البلازما والدماغ الكلي الدماغ لكلا المجموعتين. كشفت بيانات البحث عن انخفاض في فعالية الأتروبين العلاجية من التسمم المحدث بمثبطات خميرة الكولين أستراز (الديازينون) في أفراخ الدجاج التي تعاني من الاجهاد التأكسدي المحدث ببيروكسيد الهيدروجين وينصح هنا بزيادة جرعة الأتروبين لتعزيز الكفاءة العلاجية من التسمم في هذه الحالة.

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