

## The protective role of *Datura stramonium* leaves ethanolic extract against acute carbaryltoxicity in rats

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### Abstract

**Background and objective:** Two experiments were designed and performed in the present study in order to investigate the protective role of *Datura stramonium* leaves ethanolic extract against acute carbaryl toxicity in rats.

**Methods:** The first experiment was designed to study the acute toxicological effects of *Datura stramonium* crude ethanolic extract and carbaryl in rats by determining the oral median lethal dose (LD<sub>50</sub>) using up and down method. The second experiment was isobolographic analysis that used to determine the sort of interaction of the plant extract & carbaryl alone & in combination.

**Results:** The calculated median lethal dose (LD<sub>50</sub>) was (3185.25) mg/kg B.W. for ethanolic extract of *Datura stramonium* leaves & (328.5) mg/kg B.W. for carbaryl insecticide. The clinical signs were observed daily during the course of experiment, animal with toxic dose of carbaryl showed mainly cholinergic effect symptom, while those with toxic dose of *Datura stramonium* extract showed mainly anticholinergic effect symptom. The result of isobolographic analysis showed that the sort of interaction was highly antagonism. There was increase in the combined LD<sub>50</sub> of carbaryl & *Datura stramonium* extract nearly double that of each one alone, this was due to the plant contain high level of tropane alkaloids that abolish carbaryl cholinergic toxic effect by blocking the muscarinic receptors of parasympathetic nerve ending.

**Conclusion:** *Datura stramonium* leaves extract shows highly protective effect against carbaryl cholinergic toxic effect in acute exposure in rats.

**Keywords:** *Datura stramonium*, carbaryl, insecticide, anticholinergic, isobolographic analysis.

### 1. Introduction

One of the most important medicinal plants is *Datura* (thorn apple), which is an annual plant belongs to the family of Solanaceae. *Datura* plant is an important medicinal plant as it is a well-known source of different phytochemicals, it is distributed throughout most of the parts of the world [1]. It is indigenous to Caspian region and in United States, South America, France, Germany, Hungary and Middle East [2]. Traditionally it has an important medicinal value throughout the world, its leaves and seeds are used in different treatment recipes. This plant is rich in alkaloids which induce a stimulation of central nervous system and depression of the peripheral nerves typical for a

parasympathetic. Its medical effects characteristics include spasmolytic, antispasmodic, anticholinergic and narcotic [3]. The leaves of *Datura stramonium* are mixed with mustard oil for treatment skin disorder. Juice of flower petals is used in ear pain. Seeds are used as purgative, in cough, fever and asthma. Seeds are also used for smoking for its narcotic action [4]. Also *Datura stramonium* is widely growing plant and well known to have potent pharmacological activity with a great utility and usage in folk medicine [5,6]. The secondary metabolites of *Datura stramonium* are highly effective against different types of diseases such as antidiabetic, antiviral [7].

The primary biologically active substances in *Datura stramonium* are the alkaloids atropine and scopolamine. Atropine has been used in treating Parkinson' disease, peptic ulcers, diarrhea, and bronchial asthma[8].

Pesticides are a broad range of substances most commonly used for control agricultural pests and in public health programs to control the transmission of vector borne diseases. Nowadays, hundreds of active ingredients and thousands of formulations are available in the world market[9]. About 1.5 million tonnes of pesticides are manufactured every year[10,11]. Carbaryl a major active ingredient of many commercial formulations of pesticide, is extensively used for pest control, such as plant hoppers, onion thrips and fruit flies [12,13]. However, carbaryl residue in food, water and environment has severe threat to human and animal health due to its high toxicity to acetylcholinesterase (AChE), which is essential for the central nervous system of human and animal[14,15].

The primary mechanism of action of carbaryl involves a direct effect on the nervous system cause inhibition of acetylcholinesterase (AChE) activity. Carbaryl is moderately to very toxic and is labelled with a WARNING signal word. It can produce adverse effects in humans by skin contact, inhalation or ingestion. The symptoms of acute toxicity are typical of the other carbamates. Direct contact of the skin or eyes with moderate levels of this pesticide can cause burns. Inhalation or ingestion of very large amounts can be toxic to the nervous and respiratory systems resulting in nausea, stomach cramps, diarrhea and excessive salivation, other symptoms at high doses include sweating, blurring of vision, incoordination, and convulsions[16].

## 2. Materials and Methods

Fresh *Datura stramonium* leaves were collected and then dried in room temperature at shade. The dried leaves were crushed by a laboratory blender. The plant classification was done in Ministry of Agriculture/ State Board for Seeds Testing and Certification (S.B.S.T.C) in Abu Graib /Baghdad.

Alcoholic solvent extraction of the *Datura stramonium* was carried out by using ethanol (95% ethyl alcohol) which is considered as a very effective in extracting the active ingredients of the plant according to method described by Uma Reddy[17]. This was done by using Soxhlet apparatus, yield extract was calculated and the final extract was kept frozen at  $-20^{\circ}\text{C}$  until use. Carbaryl (Problte, Spain) was used in this study, choosing of doses was based on determining of acute median lethal dose ( $\text{LD}_{50}$ ) orally for *Datura stramonium* leaves ethanolic extract and carbaryl according to up and down method [18].

Thirty male Wister albino rats about three months of age and with body weight ranged between 300 – 350 g. were used to perform the three experiments of the present study. Rats were housed in plastic cages  $20 \times 50 \times 75$  c.m dimensions, placed in a special housing room belong to the Department of Physiology and Pharmacology /College of Veterinary Medicine University of Baghdad for adaptation.

## 3. Results

### 3.1 Acute Toxicity Study Oral $\text{LD}_{50}$ of *Datura stramonium* Ethanolic Extract

Determination of acute toxic median lethal dose ( $\text{LD}_{50}$ ) in albino rat was done by up and down method, the value of  $\text{LD}_{50}$  was 3185.25 mg/kg B.W, and it was calculated according to the following equation result: Table (1).

$$\text{LD}_{50} = \text{xf} + \text{kd}$$

$$= 3000 + (0.741) \times 250 = 3185.25 \text{ mg/kg B.W}$$

**Table (1): Calculate of  $\text{LD}_{50}$  for *Datura stramonium* ethanolic extract leaves by up and down method**

Range of doses	2250 - 3500 mg /kg B.W
Decrease or increase in dose	250 mg /kg B.W
Death or survival of animal after 24 hours.	OOO <u>OX</u> OXO
Value of (K) table	0.741
Last used dose ( xf)	3000 mg /kg
Value of $\text{LD}_{50}$	3185.25 mg/kg B.W

O: survival, X: death

The oral  $\text{LD}_{50}$  of *Datura* extract considered as moderately toxic as its toxicity rate is (3), appendix (1). The symptoms indicate the anticholinergic effect accordingly with the dose,

since it include symptom (depression, restlessness, dry mouth, thirst, blurred vision, anorexia, convulsion, constipation, mydriasis). Table (2).

**Appendix (1): Table of Toxicity Rating Chart**

PROBABLE L ETHAL ORAL DOSE FOR HUMANS		
Toxicity Rating or Class	Dosage	For Average Adult
1. practically nontoxic	>15 g /kg	More than 1 quart
2. Slightly toxic	5 - 15 g /kg	Between pint and quart
3. Moderately toxic	0.5 – 5 g/kg	Between ounce and pint
4. Very toxic	50 – 500 mg /kg	Between teaspoonful and ounce
5. Extremely toxic	5 - 50 mg /kg	Between 7 drops and teaspoonful
6. Super toxic	< 5 mg / kg	A taste ( less than 7 drops )

SOURCE: Gosselin, *et al.* (1984)[19].

**Table (2): The toxic symptoms and mortality outcome that developed according to different doses of *Datura stramonium* 95% ethanolic leaves extract in rat according to up and down method.**

Dose (mg/Kg.B.W)	Clinical signs during 24 hours	Results (O or X)
2250	Depression, anorexia, pupil dilatation.	O
2500	Depression, dilated pupils, anorexia, restlessness.	O
2750	Restlessness, rapid pulse, rapid breathing, dry mouth, thirst, anorexia.	O
3000	Restlessness, dry mouth, thirst, blurred vision, anorexia, restlessness, convulsion, constipation.	O
3250	Convulsion, dry mouth, restlessness, constipation, blurred vision, anorexia, paralysis, coma, death through 24hours.	X
3000	Restlessness, dry mouth, thirst, anorexia, blurred vision, restlessness, convulsion.	O
3250	Convulsion, dry mouth, constipation, restlessness, blurred vision, seizures, paralysis, coma, death through 24hours.	X
3000	Restlessness, dry mouth, thirst, constipation, anorexia, blurred vision, restlessness, convulsion.	O

O: survival,X: death

**3.2 Acute Toxicity Study of Carbaryl Rat-Oral LD<sub>50</sub>:** Determination oral LD<sub>50</sub> of acute toxic median lethal dose (LD<sub>50</sub>) in male albino rat was done by up and down method, the value of LD<sub>50</sub> was 328.5

mg/kg B.W, which was calculated according to the following equation result, Table (3).

$$LD_{50} = xf + kd = 350 + (-0.860) \times 25 = 328.5 \text{ mg/kg B.W.}$$

**Table (3): Calculate of LD<sub>50</sub> for carbaryl orally by up and down method**

Range of doses	225 - 350 mg /kg B.W
Decrease or increase in dose	25 mg /kg B.W
Death or survival of animal after 24 hours.	OOOOXOOX
Value of (K) table	- 0.860
Last used dose ( xf)	350 mg /kg
Value of LD <sub>50</sub>	328.5 mg/kg B.W

O: survival, X: death

The oral LD<sub>50</sub> of carbaryl considered as very toxic according as toxicity rate is (4), appendix (1), the symptom indicate the cholinergic effect of carbaryl due to its reversible inhibition of

acetylcholinesterase, the symptoms manifested by (anorexia, salivation, diarrhea, muscle tremors, CNS depression, lacrimation, twitching, arching back, nasal discharge).Table (4).

The toxic symptoms of carbaryl and mortality outcome develop were listed in table (4):

**Table (4): The toxic symptoms and mortality outcome that developed according to different doses of carbaryl in rat according to up and down method**

Dose (mg/Kg. B.W)	Clinical signs during 24 hours	Results (O or X)
225	Anorexia, salivation, muscle tremors, CNS depression.	O
250	Muscle tremors, anorexia, salivation, muscle tremors, diarrhea, CNS depression.	O
275	Excessive tearing and salivation, twitching, back arching, nasal discharge, diarrhea, anorexia.	O
300	Excessive tearing and salivation, diarrhea, twitching, pin-point pupils, C.N.S. depression, anorexia, nasal discharge, back arching.	O
325	Excessive tearing and salivation, C.N.S. depression, anorexia, diarrhea, twitching, pin-point pupils, back arching, nasal discharge, death through 24hours.	X
300	Excessive tearing and salivation, C.N.S. depression, diarrhea, anorexia, back arching, twitching, pin-point pupils, nasal discharge.	O
325	Excessive tearing and salivation, back arching, C.N.S. depression, anorexia, muscle tremors, twitching, pin-point pupils, diarrhea, nasal discharge.	O
350	C.N.S. depression, anorexia, archingback, excessive tearing and salivation, muscle tremors, twitching, nasal discharge, diarrhea, death through 24hours.	X

O: survival, X: death

**3.3 Study the Interaction between *Datura Stramonium* Extract & Carbaryl Toxic Doses:** The result of isobolographic analysis by using the LD<sub>50</sub>

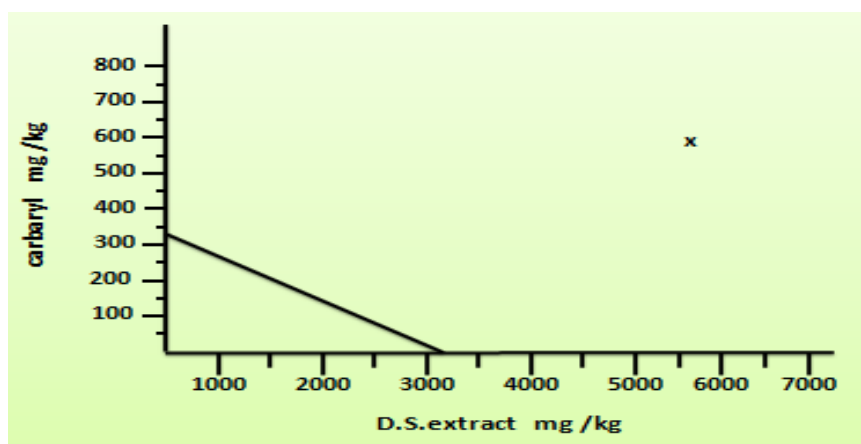
dose of both D.S. ethanolic extract & carbaryl alone & in combination are listed in table (5) & figure (1).

**Table (5):- Isobolograph analysis of both carbaryl & *Datura Stramonium* ethanolic extract LD<sub>50</sub> alone & in combination.**

parameters groups	Range of doses mg /kg body weight	Initial dose mg /kg body weight	final dose mg /kg body weight	Number of animal	Result after 24 hours	LD <sub>50</sub> mg /kg
<i>Daturastramonium</i> extract	2250 - 3500	2250	3500	8	OOOOXOXO	3185.25
Carbaryl	225 - 350	225	350	8	OOOOXOOX	328.5
D.S. extract + carbaryl	4250-5500 + 450 - 575	3000 + 325	5500 + 575	8 + 8	OOOOXXOO	5595.25 + 584.525

O: survival, X: death

**Figure (1):- Isobolograph analysis of both carbaryl & *Datura Stramonium* ethanolic extract LD<sub>50</sub> alone & in combination**



#### 4. Discussion

These results indicate the dominant anticholinergic effect of plant extract due to its a tropane alkaloid like atropine, scopolamine & hyoscyamine. This results are in agreement with Khalili and Atyabi[20], whose studied the LD<sub>50</sub> of alcoholic *Datura stramonium* seeds extract in male rats. According to his results, the median lethal dose was 2300 mg/kg., this dose was chosen to be the base of doses for acute toxicity study.

This variance in LD<sub>50</sub> values with the results of this study because the variance in route of administration of extract of *Datura stramonium* or may be the seeds have higher levels of tropane alkaloids than leaves.

The toxic symptoms of *Datura stramonium* and mortality outcome listed in table (2). These symptom were nearly similar to that reported by other researcher on *Datura stramonium* toxicity in rat that include:- dizziness, dry mouth, flushed skin, palpitations, drowsiness, tachycardia, blurred vision, mydriasis, hyperthermia, disorientation, agitation, delirium, urine retention, hypertension and coma[21].

In view of the excitatory symptoms of carbamate poisoning and since the acute toxicity of the carbamate pesticides correlates well with their potency to inhibit AChE[22], it is unlikely that the inhibitory effects on nicotinic ACh receptors (nAChRs) contribute to the acute poisoning syndrome.

However, some carbamates may also induce a variety of neurological changes at dose levels producing no overt signs of acute toxicity[22]. Carbaryl reported is very toxic when ingested by male and female rats. The oral LD<sub>50</sub> in male and female rats is 302.6 mg/kg and 311.5 mg/kg, respectively[23]. While U.S. National Library of Medicine detected the oral LD<sub>50</sub> of carbaryl ranges from 250 to 850 mg/kg in rats, and from 100 to 650 mg/kg in mice[24]. These were in agreement with our result.

The intersection of combined LD<sub>50</sub> showed that sort the interaction is highly antagonism since there was increase in the LD<sub>50</sub> of combined carbaryl & *Datura stramonium* extract doses nearly to the double that of each one alone, making the intersection point shifting far away to the right from the triangle of both LD<sub>50</sub> alone doses, this was because, the extract have tropane alkaloids such as (Atropine, Scopolamine and Hyoscyamine), that antagonized carbaryl toxicity through blocking the muscarinic receptors and so depriving the accumulated acetylcholine at the synapse from binding with its receptor & there by abolish the over stimulation cholinergic effect manifested by carbaryl

toxicity caused by its reversible inhibition effect of acetylcholinesterase, and because *Datura stramonium* contains atropine and other anticholinergic compounds, it is a useful remedy for the central cholinergic symptoms of carbaryl poisoning. Bania *et al*[25] determined the beneficial effect of *Datura* seed extracts following a severe organophosphate (OP) poisoning. According to their experiment, *D. stramonium* seeds were heated in water to make 2 mg/mL atropine solution and administered to male rats as a single intraperitoneal injection 5 min before the subcutaneous injection of 25 mg/kg of dichlorvos. Pre-treatment with *Datura* seed extracts significantly increased survival in a rat model of severe OP poisoning.

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