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Experimental Ivermectin Poisoning in Rabbits with Trial For Treatment

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Abstract

HE aim of this study was to investigate the effects of ivermectin poisoning on the brain and some trials and demonstrate the uses the nikethamide or neostigmine as a new method for treatment of ivermectin poisoning with histopathological study. The most important results obtained are: the lethal dose for ivermectin (LD50%) is 67.37 mg/Kg B.W in rabbits, The toxic dose is 33.7 mg/Kg B.W. in rabbit. The pilot study shows that recommended dose for Nikethamide is 0.07 mg/Kg B.W. in rabbits and recommended dose for Neostigmine is 0.05 mg/Kg B.W. in rabbit. The brain histopathological study of G1 group didn't showed any pathological changes, meanwhile the G2 group showed sever pathological changes characterized by Leukoencephalomalacia and perineural and perivascular edema with sever gliosis (satillatosis), and limited pathological lesions recorded in G3. The G4 showed mild histopathology changes as perivascular edema and inflammatory cell aggregation within blood vessels also astrocyte hypertrophy recorded, the histopathological study of G5 group showed congested blood vessel with sever encephalomalacia, microglia hyperplasia and neuronal chromatolysis and neuronophagia. The lesions in brain were directly related to the type of treatment.

We conclude that neostigmine and Nikethamide have detoxifying activity of ivermectin toxicity each alone but Nikethamide is the most important one and has the best safety margin while the combination of neostigmine and Nikethamide is not safe.

Keywords: Ivermectin, Nikethamide, Neostigmine, rabbit, Histopathology

Introduction

Ivermectin has been used as an endectocide in several species, including humans, for the last 30 years [1,2]. It was the first avermectin to be utilised in veterinary medicine. One member of the avermectin family of medicines is ivermectin, which is used to treat parasites. Approximately 80% of ivermectin is an avermectin B1a (AB1a) analog and 20% is an avermectin B1b (AB1b) analog. Two of the four avermectins made by the actinomycete Streptomyces avermitilis are these chemicals [3,4]. For the control of both external (ecto) and internal (endo) parasites in sheep and goats, the medication ivermectin is employed [5]. Ivermectin alleviates symptoms of onchocerciasis, as well as those of ascaraisis, trichuriasis, filariasis, entrobiasis, and scabies in humans [6]. It is a member of the avermectin group of macrolide antibiotics and an agonist for the inhibitory GABA. It may be injected, applied topically, or taken orally [7]. This medicine blocks the conduction of nerve impulses by binding to GABA-gated chloride and invertebratespecific glutamate-gated anion channels in peripheral neuromuscular synapses [8].

The GABA receptors in mammals are primarily located in the CNS, where they are protected by the blood brain barrier, but in arthropods and nematodes, they are located in the peripheral nervous system, specifically at the neuromuscular junction, which is

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why it is useful as an anthelminthic. Endo- and ectoparasites' flaccid paralysis and inhibition of eating are caused by stimulation of GABA receptors [2, 9].

While most mammals may safely use ivermectin, there are a small number of species that are very susceptible to Severe adverse effects on the central nervous system, including depression, coma, and death, which may manifest as ivermectin poisoning [2]. Humans are among the many animals that have reported harmful effects. When administered dosages four to eight times higher than the permitted dosage, horses, cattle, sheep, pigs, and rabbits exhibit neurotoxic symptoms such as depression, ataxia, stiffness, and decreased eyesight [1].

The lack of P-glycoprotein in the capillary endothelium of the nervous system is thought to be the cause of these symptoms in animals [2]. Pglycoprotein is an efflux pump that helps keep certain medications out of the central nervous system by forming a blood-brain barrier [10]. Particularly in CF-1 mice and collie dogs, ivermectin has had serious adverse effects on the central nervous system. When exposed to the same amount as other dogs, sensitive collies respond at a concentration as low as 1/200th of the lethal level [2].

The activity of drug efflux transporters causes ivermectin, which is particularly lipophilic, to typically have very limited penetration of the blood brain barrier. Ivermectin is one of many medications that are better absorbed and exposed to the brain in people whose genes are either defective or disrupted. There have been reports of treatment having minor impacts on immunological function [12,13] and behavior [11]. Mydriasis, depression, ataxia, recumbency, and mortality are the symptoms of ivermectin toxicosis in animals [3,4,14].

There is extensive therapeutic usage of neotiathamide with the purpose of enhancing CNS and cardiovascular system function. It is crucial to examine the effects of nikethamide on the energy metabolism of animal tissues since this substance is an analogue of nicotinamide, the catalytically active group of the nicotinamide-adenine dinucleotide (NAD) molecule. Researchers have shown that nikethamide blocks the respiratory chain of mitochondria from transporting hydrogen and electrons, which impacts the oxidative metabolism of cardiac tissue. While it is unclear from the references if this applies to human subjects, it is stated in [15-18] that nikethamide is active when taken orally. In a study conducted on rabbits, the amount of convulsant medication administered orally was five times higher than the dose administered intravenously [19]. According to [20], when given orally in modest dosages, it has a profound effect on animals used in experiments. When it comes to treating cardiac dyspnea, coronary artery disease, and peripheral

circulatory failure, oral nikethamide has been the subject of several investigations, but the published results have been equivocal [21,22].

An inhibitor of the GABAA receptor, a ligandgated ion channel of the main inhibitory neurotransmitter γ -aminobutyric acid (GABA), is known as a GABAA receptor negative allosteric modulator (NAM) [23,24]. Medications that block the GABAA receptor are quite similar to these. In terms of their functional effects, GABAA receptor NAMs are opposed to benzodiazepines, barbiturates, and alcohol, which are examples of GABAA receptor PAMs. A number of side effects, including as anxiety, neurotoxicity, and convulsions, may be produced by non-selective GABAA receptor NAMs [23,24].

As an anticholinesterase drug, neostigmine competes with acetylcholine for binding to acetylcholinesterase, therefore inhibiting reversibly the breakdown of acetylcholine. The outcome is an increase in the concentration of acetylcholine at cholinergic synapses, which causes its effects to be both extended and compounded [25]. It works by blocking acetylcholinesterase, which makes acetylcholine (Ach) more effective. An allencompassing cholinergic response may therefore be generated by it [25].

Among neostigmine's side effects include miosis, bradycardia, increased skeletal and intestinal muscle tone, constriction of the airways, and stimulation of the sweat and saliva glands. The drug is most commonly prescribed for its cholinomimetic effects on skeletal muscle, though it can also increase smooth muscle activity to a lesser degree. From 47 to 60 minutes after intravenous injection, its half-life begins [26]. Reversing neuromuscular blockade with neostigmine requires careful ventilation and the maintenance of a patent airway until the patient's respiration returns to normal [25].

Since ivermectin does not yet cross the bloodbrain barrier and GABA-mediated nerves occur in the central nervous system (CNS), mammals are less vulnerable to the harmful effects of ivermectin. Brain levels peak two to five hours after injection, and relatively large dosages penetrate the blood-brain barriers [27].

The aim of study was to study the effect of ivermectin poisoning on tissue of brain as histopathological changes to demonstrate the uses the nikethamide or neostigmine's as a new method for treatment ivermectin poisoning with histopathological study.

Material and Methods

Animals

We purchased twenty male rabbits (Lepus cuniculus) from a local vendor in Fallujah city,

Anbar Province, Iraq. Their weight ranged from 1200 to 2000 grams, and they were 8 to 12 months old. The rabbits were kept in wire silk cages at the animal house of the University of Fallujah's Veterinary Medicine College in controlled conditions, with a temperature of 25 ± 3 °C and a relative humidity of $50 \pm 5\%$. Each cage had four rabbits. For a month, the bunnies were watched closely.

Experimental Design

The study was carried out in compliance with international ethical guidelines for the use of laboratory animals and with the agreement of the College of Veterinary Medicine/University of Fallujah.

The rabbits were divided into five groups (4 rabbits in each group) as the following:

- 1- Group (A): Negative control, given the food and water.
- 2- Group (B): Positive control: Induce Toxicity by Ivermectin with a single dose 33.7 mg/Kg B.W by Subcutaneous injection (S/C injection).
- 3- Group (C): Induce Toxicity by Ivermectin with a single dose 33.7 mg/Kg B.W by S/C and then treated with a single dose 0.07 mg/kg B.W of Nikethamide by Intraperitoneal injection (IP injection).
- 4- Group (D): Induce Toxicity by Ivermectin with a single dose 33.7 mg/Kg B.W, S/C and then treated with a single dose 0.05 mg/kg B.W of Neostigmine methylsulfate by Intraperitoneal injection (IP injection).
- 5- Group (E): Induce Toxicity by Ivermectin with a single dose 33.7 mg/Kg B.W S/C and then treated with a single dose 0.05 mg/kg B.W Nikethamide

+ single dose 0.07 mg/kg B.W of Neostigmine methylsulfate by Intraperitoneal injection (IP injection).

Acute toxicity study (single lethal dose LD₅₀)

There were a total of six rabbits used in the study. Two rabbits in the first group were given a single lethal injection of 50 mg/kg body weight of ivermectin; two rabbits in the second group received 60 mg/kg body weight of ivermectin; and two rabbits in the third group were given a single lethal injection of 70 mg/kg body weight of ivermectin. The LD₅₀ for each group was determined using the up and down method [28].

Dixon method

The first dosage for this experiment is to be set at "the toxicologist's best estimate of the LD50," and the animals are to be dosed individually every 24 hours. In accordance with a predetermined dose progression factor, the dosage is reduced after each death and raised after each survival. After three more animals are tested following the same pattern of dose adjustment, testing is concluded if death occurs after an initial direction of increasing doses by 10-20% or if survival occurs after an initial direction of reducing dosage by the same ratio due to constant variables. We determine the LD₅₀.

Dosages and dosing

All drugs in question were calculated according to the body weight of animal as mg/Kg.BW. They were administered Ivermectin Subcutaneously with a single dose 33.7 mg/Kg B.W with dose volume 3.37 ml/1 Kg.BW of each Rabbit by calculating and fitting all their concentrations for all experiments in our study. They were administered Nikethamide by Intraperitoneal injection with a single dose 0.07 mg/Kg B.W with dose volume 0.07 ml/1 Kg.BW of each Rabbit. They were administered Neostigmine methylsulfate by Intraperitoneal injection with a single dose 0.05 mg/Kg B.W with dose volume 0.5 ml/1 Kg.BW of each Rabbit.

Electrometric Measurement of Plasma Cholinesterase Activities

Esterase level (AchE) AchE test was done by using (Michel method) which is based on the change of pH of media by using (pH meter). It's done by adding 3 ml distilled water and 3 ml of barbitalphosphate buffer with pH 8.1* to all test tubes that contain plasma and to the blank, leave it for one hour at 25 °C, then the (pH1) is measured, then by adding (0.1) of aqueous solution of acetylthiocholine iodide (0.7%) as a substrate to all test tubes, that incubated at 37Co for 20 minutes. At the end of the incubation period, the (pH2) of all tubes was measured [29,30]. The enzyme activity was calculated as follows:

 ΔpH of AchE (ΔpH \20 minute) =(pH1-pH2)- ΔpH of blank

*pH 8.1 buffer consisted of 1.237g sodium barbital+0.63g of potassium dihydrogen phosphate + 35.07 g of sodium chloride, all dissolved in 1 liter of distilled water.

And then using the following equation to calculate the percentage of AchE inhibition:



Histopathological study

Samples were taken from the Brain for histopathological study. The tissue sample was kept in 10% natural puffer formalin till the time of processing. Slices thickness was 4 μ . Then stain with routine stain Hematoxylin and eosin (H&E) [31; 32].

Statistical analysis

The data are presented as the mean \pm SE. We used SPSS version 24.00 to conduct the analysis. Statistical significance was determined using a one-way ANOVA with a p-value less than 0.05. The means were compared using LSD multiple range tests.

Results and Discussion

Acute toxicity

This table (1) shows how to calculate the lethal dose and later determine the toxic dose in the Rabbits and is calculated according to up & down method [27].

In table 2 shows how to choose the dose of Nikethamide dosed Intraperitoneal injection to Rabbit for reducing convulsion and then choose 0.07 mg/Kg B.W

In table 3 shows how to choose the dose of Neostigmine methylsulfate dosed Intraperitoneal injection to Rabbits then choose 0.05 mg/Kg B.W according to pilot study.

The Pupil of eye measure in rabbits indicated significant differences between groups; After 3 Minutes showed significant increase (P<0.05) in groups G2, G3, G4 and G5, while after one hours G5 heart rates return to normal and G3 decrease significantly without significant difference as compared with G1 while G4 decrease significantly but still higher than G1. The results of Pupil of eye measure in G2 increased significantly (P<0.05) due to stimulation of gamma-Aminobutyric acid and inhibit the parasympathetic nervous system (decrease level of Acetylcholine (ACh)) and stimulation of sympathetic nervous system by the effect of Ivermectin, while treated groups have a reverse the effect of ivermectin with the different degree [30-32]. The actual mechanisms of nikethamide are blocks ionotropic GABA receptors [33]

Significant differences between the various treatment groups at (P < 0.05) are indicated by the different capital letters.

The various little letters indicate that there are significant differences between various times at a level of significance of P < 0.05.

Heart rates of rabbits indicated significant differences (P<0.05) between groups; After 3 Minutes showed significant increase (P<0.05) in groups G2, G3, G4 and G5, while after one-hour G3 heart rates return to normal and G4 decreased significantly but still higher than normal range while G5 decrease significantly below the normal range. The results of Heart rates in G2, G3, G4 and G5 after three minutes increased significantly due to

stimulation of gamma-Aminobutyric acid and inhabit the parasympathetic nervous system (decrease level of Acetylcholine (ACh)) and stimulation of sympathetic nervous system by the effect of Ivermectin after three minutes, while treated groups after one hours reverse the effect of Ivermectin with the different degree [30,31,32]. According to studies examining nikethamide's impact on cardiac oxidative metabolism, this chemical blocks the respiratory chain of mitochondria from transporting hydrogen and electrons [15,16,17]. gamma-aminobutyric acid (GABA) is the main neurotransmitter responsible for lowering neuronal excitability in the central nervous system of developing mammals. The control of muscular tone in humans is also directly regulated by GABA [34].

The results of Respiratory rates of rabbits indicated significant differences (P<0.05) between groups; After 3 Minutes show significant increase in groups G2, G3, G4 and G5, while after one hours G3 heart rates return to normal and G4 decrease significantly but still higher than normal range while G2 and G5 decrease significantly below the normal range. The results of Heart rates in G2, G3, G4 and G5 after three minute increased significantly due to stimulation of gamma-Aminobutyric acid and inhabit the parasympathetic nervous system (decrease level of Acetylcholine (ACh)) and stimulation of sympathetic nervous system by the effect of Ivermectin after three minute, while treated groups after one hours reverse the effect of Ivermectin with the different degree, , in G2 and G5 Respiratory rates decreased significantly below the normal range due to fatigue in respiratory muscle [30,31,32]. Nikethamide, a respiratory center stimulant. However, its effects on the central nervous system and medullary respiratory center [35].

Cholinesterase inhibition in rabbits indicated decreased level of Cholinesterase inhibition in G2, and significant decrease in G5. Cholinesterase inhibitory effect might be due to acetylcholine level and its level decrease due to stimulation of gamma-Aminobutyric acid by the effect of Ivermectin, An overabundance of the ivermectin molecule in the central nervous system (CNS) causes an increase in gamma-aminobutyric acid (GABA) activity, which in turn causes presynaptic neurons to release more GABA and improve its postsynaptic binding to its receptors. leading to ivermectin poisoning. Hyperpolarization of the cell membranes occurs as a result of an increase in the influx of chloride ions inside the neurons. Consequently, this leads to a widespread obstruction of the CNS stimulation systems, which in turn disrupts normal neurological activities [31]. In G4 Acetylcholine level is still below the normal level due to the mechanism of action of neostigmine by enhances the transmission

of acetylcholine signals not by increase release of Acetylcholine and that lead to dissimilar results and doubtful to use it as antidote [30]. The actual mechanisms of nikethamide were anticholinesterase and blocks ionotropic GABA receptors [33] It is therefore capable of producing a generalized cholinergic response [25].

The brain histopathological study of G1 group not showed any pathological changes (fig. 1), meanwhile the G2 group showed sever pathological change characterized by Leukoencephalomalacia and perineural and perivascular edema with sever gliosis (satillatosis) (Fig.2 and 3), and limited pathological lesion recorded in G3 as seen in (Fig. 4). The G4 showed mild histopathology changes as perivascular edema and inflammatory cell aggregation within blood vessels also astrocyte hypertrophy recorded (Fig. 5), the histopathology study of G5 group showed congested blood vessel (arrow) with sever encephalomalacia (arrowhead), microglia (double hyperplasia arrow) and neuronal chromatolysis and neuronophagia (Figs. 6, 7, 8). The lesions in brain were directly related to the type of treatment.

Mahmoud *et al.* [38] and Shoeb [39], who also found brain lesions in G2, found same findings. According to Ming *et al.* [40], pigeon brain tissues exposed to subchronic dosages of ivermectin showed pathological alterations such as neuronal degeneration and necrosis.

Conclusion

We Conclude the following:

- 1-The most important results arrived at are lethal dose LD50% is 67.37 mg/Kg B.W for rabbits.
- 2-The toxic dose is 33.7 mg/Kg B.W. in rabbits.
- 3-Nikethamide has detoxifying activity for over dose of ivermectin while neostigmine has less detoxifying activity for the same purpose.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest

None

Authors contributions

Mohammed Mosleh Shwaish: Practical work

Mustafa Salah Hasan: Manuscript writing and editing. Ahmed Sami Jarad: Histopathological work and reading Falah Muosa Kadhim AL-Rekabi: statistical analysis.

TABLE 1. The result of up and down method for calculation LD50 of Ivermectin, after acute S/C injection in Rabbit.

Drug	Initial dose mg/Kg B.W	Last dose mg/Kg B.W	No of animals	Different between doses	Outcome	LD 50 mg/Kg B.W
Ivermectin	50	60	6	10	0 0 X 0 X 0	67.37
O = live						

TABLE	2. The	results	of pilot	study	of	Nikethamide	dosed	Intraperitoneal	injection	to	Rabbit	for
	reducin	ng convu	ılsion									

Dose mg/Kg.BW	Number of poisoned animals	Number of recovered animals with 24 hours
0.04	2	0
0.05	2	1
0.06	2	1
0.07	2	2

 TABLE 3. The results of pilot study of Neostigmine methylsulfate dosed Intraperitoneal injection to Rabbit for reducing convulsion

Dose mg/Kg.BW	Number of poisoned animals	Number of recovered animals with 24 hours
0.02	2	0
0.03	2	1
0.04	2	1
0.05	2	2

TABLE 4. The results of Pupil of eye measure by millimeter in all groups at different times .

	Pupil of eye measure by millimeter after different time				
Groups of Animals	After 3 Minutes	After 10 Minutes	After 30 Minutes	After 1 hours	
G1- Animals are normal	0.65±0.06 ^C	0.65±0.06 ^C	0.65±0.06 ^C	$0.65 {\pm} 0.05^{C}$	
G2-Animals are given ivermectin (33.7) mg/Kg B.W. only	1.47±0.09 ^A	1.65±0.06 ^A	1.70±0.04 ^A	1.68±0.05 ^A	
G3- Animals are given ivermectin (33.7) mg/Kg B.W and then given nekithmid (0.07) mg/Kg B.W only	$1.05{\pm}0.06^{Ba}$	$0.60{\pm}0.1^{Cb}$	$0.73 {\pm} 0.04^{Cb}$	$0.73 \pm 0.04 \text{BCb}$	
G4- Animals are given ivermectin (33.7) mg/Kg B.W and then given neostigmine (0.05) mg/Kg B.W only	1.43±0.08 ^{Aa}	1.20±0.09 ^{Bab}	$1.08{\pm}0.04^{Bbc}$	$0.90{\pm}0.04^{\rm Bb}$	
G5- Animals are given ivermectin (33.7) mg/Kg B.W and then given Nekithmid (0.07) + neostigmen (0.05) mg/Kg B.W	$0.83{\pm}0.08B^{Ca}$	$0.30{\pm}0.04^{\text{Db}}$	0.33±0.06 ^{Db}	0.63±0.34 ^{Ca}	

LSD=0.24

Significant differences between the various treatment groups at (P < 0.05) are indicated by the different capital letters.

The various little letters indicate that there are significant differences between various times at a level of significance of P < 0.05.

	Heart rates measure after different time				
Groups	After 3 Minutes	After 10 Minutes	After 30 Minutes	After 1 hours	
G1: Animals are normal	79.75±0.85 ^C	79.0±1.68 ^D	77.75±1.11 ^C	76.00±1.47 ^C	
G2: Animals are given ivermectine (33.7) mg/Kg B.W. only	122.75 ± 5.85^{Bb}	137.25±4.26 ^{Aa}	143.25±2.36 ^{Aa}	143.50±1.55 ^{Aa}	
G3: Animals are given ivermectine (33.7) mg/Kg B.W and then given nekithmide (0.07) mg/Kg B.W only	111.25±3.83 ^{Ba}	82.0 ± 2.48^{CDb}	78.0±1.47 ^{Cb}	76.50±1.71 ^{Cb}	
G4: Animals are given ivermectin (33.7) mg/Kg B.W and then given neostigmine (0.05) mg/Kg B.W only	135.25±3.56 ^{Aa}	129.25±0.85 ^{Ba}	125.75±4.04 ^{Ba}	99.25±1.85 ^{Bb}	
G5: Animals are given ivermectin (33.7) mg/Kg B.W and then given Nekithmide (0.07) + neostigmine (0.05) mg/Kg B.W	121.25±2.98 ^{Ba}	91.50±2.84 ^{Cb}	79.50±4.11 ^{Cc}	52.50±17.91 ^{Dd}	

TABLE 5. The results of heart rates measure in all groups at different times .

LSD=11.59

Significant differences between the various treatment groups at (P < 0.05) are indicated by the different capital letters.

The various little letters indicate that there are significant differences between various times at a level of significance of P < 0.05.

TABLE 6. The results of Respiratory rates measure in all groups at different times

	Respiratory rates measure after different time				
Groups	After 3 Minutes	After 10 Minutes	After 30 Minutes	After 1 hours	
G1: Animals are normal	78.50 ± 2.50^{B}	81.0 ± 0.71^{B}	$80.75{\pm}1.31^{AB}$	78.5±1.71 ^A	
G2: Animals are given ivermectin (33.7) mg/Kg B.W. only	121.75±3.96 ^{Aa}	$92.00{\pm}1.85^{ABb}$	79.25±4.26 ^{ABc}	47.50 ± 3.71^{Bd}	
G3: Animals are given ivermectin (33.7) mg/Kg B.W and then given nekithmide (0.07) mg/Kg B.W only	90.50±2.32 ^B	80.00 ± 1.22^{B}	78.75±1.49 ^{AB}	78.75±0.94 ^A	
G4: Animals are given ivermectin (33.7) mg/Kg B.W and then given neostigmine (0.05) mg/Kg B.W only	114.50±5.60 ^{Aa}	101.75±4.75 ^{Ab}	91.0±4.72 ^{Acd}	86.75±6.99 ^{Ad}	
G5: Animals are given ivermectin (33.7) mg/Kg B.W and then given Nekithmide (0.07) + neostigmine (0.05) mg/Kg B.W	95.50±1.66 ^{Ba}	87.25±4.82 ^{Bab}	68.25±9.47 ^{Bb}	48.25±16.50 ^{Bc}	

LSD=12.66

Significant differences between the various treatment groups at ($P \le 0.05$) are indicated by the different capital letters.

The various little letters indicate that there are significant differences between various times at a level of significance of P < 0.05.

	Cholinesterase activity						
Groups	Ph1	Ph2	Δ Ph (PH1-PH2)	∆Ph of AchE/ 20min	Cholinesterase inhibitory %		
G1:Animals are normal	8.01±0.006	7.17±0.03	$0.84{\pm}0.03^{A}$	$0.74{\pm}0.03^{A}$			
G2: Animals are given ivermectin (33.7) mg/Kg B.W. only	8.06±0.02	7.33±0.07	$0.73{\pm}0.07^{AB}$	$0.63{\pm}0.07^{AB}$	13.09 % ^B		
G3: Animals are given ivermectin (33.7) mg/Kg B.W and then given nekithmide (0.07) mg/Kg B.W only	8.03±0.04	7.15±0.06	0.88±0.05 ^A	0.78±0.05 ^A	4.76 % ^A		
G4: Animals are given ivermectin (33.7) mg/Kg B.W and then given neostigmine (0.05) mg/Kg B.W only	7.94±0.22	7.13±0.12	0.82±0.23 ^A	0.72±0.23 ^A	2.38 % ^A		
G5: Animals are given ivermectin (33.7) mg/Kg B.W and then given Nekithmid (0.07) + neostigmine (0.05) mg/Kg B.W	7.81±0.09	7.40±0.11	0.42±0.04 ^B	0.32±0.04 ^B	50 % ^C		

TABLE 7.	The results of	Cholinesterase	activity in all	groups
		e nonnester ase		- S- C-PC

Significant differences between the various treatment groups at (P < 0.05) are indicated by the different capital letters.



Fig. 1. Histopathological section of Brain og G1 group showed normal histological apearance of brain (H&E stain. 40X).



Fig. 2. Histopathology section of brain of G2 group showed perineural and perivascular edema (arrow) with Leukoencephalomalacia with sever gliosis (arrowhead) (H&E stain. 20X).



Fig. 3. Histopathology section of brain of G2 group showed Leukoencephalomalacia (arrow) with sever gliosis (arrowhead) (H&E stain. 40X).



Fig. 4. Histopathological section of brain of G3 group showed mild pathological changes perivascular (arrow) and perineural edema (arrowhead)(H&E X40).



Fig. 5. Histopathological view of brain of G4 group showed perivascular edema (arrow) with inflammatory cell aggregation within blood vessels (arrowhead) and astrocyte hypertrophy (star) X40 H&E.



Fig. 6. Histopathology photograph of brain of G5 group showed congested blood vessel (arrow) with sever encephalomalacia (arrowhead) (H&E stain. 20X).



Fig. 7. Histopathology photograph of brain of G5 group showed sever encephalomalacia (arrow), microglia hyperplasia (arrowhead) (H&E stain. 40X).



Fig. 8. Histopathology photograph of brain of G5 group showed encephalomalacia (arrow), and neuronal chromatolysis and neuronophagia (arrowhead) (H&E stain. 40X).

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التسمم بالإيفر مكتين التجريبي في الأرانب مع تجربة علاجه محمد مصلح شويش¹، مصطفى صلاح حسن²، احمد سامي جراد³ و فلاح موسى الركابي⁴ ^{1*} فرع الفسلجة والادوية - كلية الطب البيطري - جامعة الفلوجة - العراق. ² فرع الطب الباطني والوقائي - كلية الطب البيطري - جامعة الفلوجة - العراق. ³ فرع علم الأمراض وأمراض الدواجن - كلية الطب البيطري - جامعة الفلوجة - العراق. ⁴ فرع الفسلجة والادوية - كلية الطب البيطري - جامعة بغداد - العراق.

كان الهدف من هذه الدراسة هو دراسة تأثيرات التسمم بالإيفرمكتين على الدماغ وبعض التجارب وبيان استخدامات النيكثاميد أو النيوستجمين كطريقة جديدة لعلاج التسمم بالإيفرمكتين مع دراسة نسجية مرضية. وأهم النتائج التي تم الحصول عليها هي: الجرعة المميتة للإيفرمكتين (%LD50) هي 67.37 ملغم/كغم من وزن الجسم في الأرانب، والجرعة السامة 3.37 ملغم/كغم من وزن الجسم. في الارنب. أظهرت الدراسة التجريبية أن الجرعة الموصى بها من نيكثاميد هي 0.00 ملغم / كغم من وزن الجسم. في الأرانب والجرعة الموصى بها للنيوستيجمين هي 0.05 ملغم/كغم من وزن الجسم في الأرانب، والجرعة السامة 7.37 التشريحية المرضية للدماغ الموصى بها للنيوستيجمين هي 20.0 ملغم / كغم من وزن الجسم. في الأرانب المرانب. لم تظهر الدراسة بتلين الدماغ الأرانب والجرعة الموصى بها للنيوستيجمين هي 20.0 ملغم / كغم من وزن الجسم. في الارنب. لم تظهر الدراسة بتلين الدماغ الأبيض والوذمة المحموعة 61 أي تغيرات مرضية، في حين أظهرت المجموعة 22 تغيرات مرضية مديدة تتميز مسجلة في .33 أظهرت مجموعة 14 أعصاب وحول الأو عية الدموية مع دباق شديد (داء الأقمار)، وآفات مرضية محدودة مسجلة في .33 أظهرت مجموعة 64 تغيرات مرضية، وي قالم الوذمة المحيطة بالأو عية الدموية مع دباق مسجلة في .33 أظهرت مجموعة 44 عصاب وحول الأو عية الدموية مع دباق شديد (داء الأقمار)، وآفات مرضية محدودة داخل الأو عية الدموية، كما تم تسجيل تضخم الخلايا النجمية، وأظهرت الدراسة التشريحية المرضية لمجموعة 65 احتفان الأوعية الدموية مع تلين دماغي شديد وتضخم الخلايا الدبقية الصغيرة وانحلال كروماتوي عصبي وبلع عصبي. وكانت الأوعية الدموية مع تلين دماغي شديد وتضخم الخلايا الدبقية الصغيرة وانحلال كروماتوي عصبي وبلع عصبي. وكانت

نستنتج أن النيوستجمين والنيكثاميد لهما نشاط إز الة السموم من سمية الإيفر مكتين كل منهما على حدة ولكن نيكثاميد هو الأهم وله هامش أمان أفضل في حين أن مزيج النيوستجمين والنيكثاميد ليس آمنًا.

الكلمات الدالة: ايفر مكتين، نيكثاميد، نيوستجمين، أرنب، هستوباتولوجي.