POTENTIATION EFFECT OF ASTONIN®-H TO CHLOROPHACINONE TOXICITY AGAINST THE ALBINO RAT, *RATTUS NORVEGICUS ALBINUS* (BERK)

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Abstract

Raising the efficiency of anticoagulant rodenticides "mineralocorticoid with a negliglible glucocortioid activity" mixed with chlorophacinone under laboratory conditions was studied. Results showed that the mortality percentage at albino rat *Rattus norvegicus albinus* increase with addition Astonin®-H (Ast.) to LD_{50} chlorophacinone, while the highest mortality were 90 and 100% at doses 70 and 90 mg/kg body weight Ast., respectively compared to 50% when chlorophacinone used alone at LD_{50}. On the other side, the low doses of chlorophacinone 1/4LD_{50} alone or when added at different doses of Ast the mortality percentage was zero except at the dose of 100 mg/kg b.wt. caused 20% mortality. The bleeding time was increased gradually with increasing exposure time up to 120h then decreased. At the same time, prothrombin time increased to double when Ast. at doses 90 and 100 mg/kg b.wt. mixed with LD_{50} of chlorophacinone compared to use it alone. On the other hand, the plasma AST and ALT increased after 48h post-treatment than 24h compared to Pre-treatment for all treatments and there were significant differences between treatments after 24 and 48h. The highest value of AST and ALT recorded at dose of LD_{50}+ 100 mg/kg b.wt. Ast. (90.35 and 31.12 U/L) after 48h of treatment, respectively.

INTRODUCTION

Anticoagulant rodenticides have been used extensively for the purpose of rodent control all over the world. These compounds have two main advantages over the older acute poisons, firstly they do not induced bait shyness (Buckle, 1994) and secondly, they are safe in their use. There are two groups of anticoagulant rodenticides i.e. hydroxycoumarin and indane-dione derivatives. Such poisons have a cumulatively effect when they consumed by the animals, without showing poisoning symptoms until the lethal quantities have been ingested. Anticoagulants reduce and cause a total loss of the clotting ability of the blood. These chemicals also cause damage to blood vessels and cause death by interfering competitive the synthesis of vitamin K in the liver. When the prothrombin level falls below a critical level, clotting

cannot occur and the tiny hemorrhages appear throughout the body and the animal bleeds to death (Brooks *et al.,* 1990).

In Egypt, anticoagulant rodenticides have long been used on a large scale to control rodents either in agriculture or public health purpose. But some problems related to efficiency of anticoagulant rodenticide appear beside apprehensively resistance. Little studies tested some potential i.e. Oshar, Neem plant extracts calciferol, L¬histidine and sulphaduinoxaline on the anticoagulant rodenticides efficiency to overcome this problem (Kandil *et al.,* 1994 and Gabr, 2006). So Astonin-H® drug can be used as synergistic for anticoagulant rodenticides, Astonin-H drug binds the mineralocorticoid receptor (aldostrone receptor) this binding (oractivation of the mineralocorticoid receptor by Fludrocortisone) in turn causes an increase in ion and water transport and thus raises extracellular fluid volume, blood pressure and decreased potassium levels. So, this study aimed to increase anticoagulant rodenticides efficiency by addition Astonin-H drug to anticoagulant bait to pave the way in rodent control programs and know some biochemical response resulted from synergistic action of Astonin-H to chlorophacinone toxicity.

MATERIALS AND METHODS

1. Tested animals:

In this work the white albino rat strain, *Rattus norvegicus albinus* (Berk) was obtained from the Egyptian Organization for Biological and Vaccine Production. The animals were carefully transported as soon as possible to the laboratory, then they were housed in metallic cages, supplied with enough food (crush maize) and water for acclimatization. The animals were observed daily for about two weeks before any experiments. During this time immature animals, pregnant females and non-healthy animals were excluded from the test. The healthy animals sexed, weighted, individually caged and given a reference number.

2. Tested Compounds:

2.1. Chlorophacinone:

- Common name: chlorophacinone
- Chemical name: 2-[2-(4-chlorophenyl)-2-phenylacetyl] indan-1,3−dione.
- Chemical Formula: $C_{23}H_{15}ClO_3$

The active ingredient of Chlorophacinone (90%EC.) was obtained from Kafr El-Zayiat Pesticides Company, Egypt. The experimental doses used in this work was 5.4 and 1.35 mg/kg body weight (Abd El-Bar, 2007), it was used as a stock stable suspension in corn oil for the purpose of orally administrated by stomach tube.

2.2. Astonin®-H drug:

- Common name: fludrocortisone

- Chemical name: 9-fluoro-11, 17-dihydroxy-17-(2-hydroxyacetyl)-10, 13-dimethyl-1, 2, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydrocyclopenta[a] phenanthren-3-one.

- Chemical Formula: $C_{21}H_{29}FO_5$

Each Astonin®-H tablet contains 0.1 mg of fludrocortisone. It was obtained from Amoun Pharmaceutical Company, Egypt as tablets. Tablets were powdered and doses of 10, 30, 50, 70, 90 and 100mg/kg body weight administrated separately or in addition to the chlorophacinone at 5.4 and 1.35 mg/kg b.wt. (Abd El-Bar, 2007) by stomach tube method.

3. Experimental design:

One hundred and eighty individuals of the adult albino white rat *R. norvegicus albinus* were used for this experiment. Acclimated rat were classified into 18 groups treated with different above-mentioned doses of Astonin-H alone or chlorophacinone at 5.4 and 1.35 mg/kg b.wt. alone or in combination of Ast.; each group contains ten rats were housed individually and allowed to fast for 12 hours before treatment. After treatment, rats supplied with enough food (crush maize) and water. A parallel control test was conducted using plain carrier. Adjusted doses of following treatment were administrated orally by stomach tube. Mortality and time to death were recorded up to 28 days after treatment. Bleeding time (B.T.) was measured before treatment and after 12, 24, 48, 72, 96, 120, 144 and 168 hours of treatment up recovery by using the method of Duke (1910).

Blood samples were collected by retro-orbital sinus puncture in clean tubes containing sodium citrate solution 3.8% (1ml citrate solution/ 9 ml blood) mixed as anticoagulant, then centerfugated at 3000 r.p.m. for 15 minutes. The supernatant plasma was removed in clean eppendorf tubes and kept under freezing condition until used for biochemical estimation [Prothrombin time (P.T.), plasma total protein, AST and ALT]. Blood samples were taken pre- and post treatment within 24 and 48 hours.

- Biochemical Determination:

- Prothrombin time (P.T.) was measured as described according to Dacie and Lewis (1984) by using reagents obtained from Diamond Company.
- Colorimetric determination of total protein content was carried out according to the method of Gornall *et al*. (1949) by using reagents obtained from Diamond Diagnostics Company.

- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes activities were determined colorimetric according to the method of Reitman and Frankle (1957) by using reagents obtained from Diamond Diagnostics Company.

4. Statistical Analysis:

The obtained results were statistically analyzed using COSTAT computer programs (2005).

RESULTS AND DISCUSSION

1. Effect on bleeding time (B.T.) and mortality percentage:

Data in Table (1) showed that the bleeding time (B.T.) increased gradually when treated rats with the LD_{50} (5.4 mg/kg b.wt.) of chlorophacinone alone after 12 h of treatment up to 72 h then decreased gradually up to 168h. While the B.T. increased after 12 h of the treatment up to 120 h then decreased gradually up to 168 h, with doses of LD_{50} + 30, 50 & 70 mg/kg b.wt. Ast. after that rats recovery was occured, also the B.T. increased gradually post 12 h of treatment up to 120h for doses of LD_{50} + 90 & 100 mg/kg b.wt. Ast. then complete mortality occured. Also, the highest mortality percentage recorded was 100% with the doses of LD_{50} + 90 & 100 mg/kg b.wt. Ast. and the lowest mortality recorded was 50% with LD50 of chlorophacinone alone. On the other hand, the highest mean lethal time value recorded was (4.61 days) with LD_{50} of chlorophacinone alone and the lowest was (3.17 days) with LD_{50} + 100 mg/kg b.wt. Ast.

Data in Table (2) cleared that the B.T. increased gradually when rats treated with $1/4LD_{50}$ (1.35 mg/kg b.wt.) of chlorophacinone alone after 12 h up to 72 h then decreased gradually up to 144 h for doses of, $1/4LD_{50}$ + 30, 50 and 70 mg/kg b.wt. Ast.. While, the doses of $1/4LD_{50}$ + 90 & 100 mg/kg b.wt. Ast. increased B.T. up to 96 h then recovery occurred. Also, the mortality percentage 20% recorded with treatments of LD_{50} + 100 mg/kg b.wt. Ast. with the mean lethal time (8 days), while the other treatments didn't record any mortality.

The data presented in Table (3) illustrated the effect of the different doses of Ast. on the bleeding time (B.T.) in albino rat, *R. norvegicus albinus*. The results showed that the B.T. increased gradually after treatment up to 24h for dose of 10 mg/kg b.wt. Ast. and up to 48h for 30 mg/kg b.wt. Ast., while up to 72h for dose of 50, 70, 90 & 100 mg/kg b.wt. Ast. and then recover occurred. Also, the all treatments didn't record any mortality.

Regarding to the previous results clear that slight effect of Astonin-H alone in rat mortality percentage, whereas the mortality percentage were 20% only when used at 100 mg/kg b.wt. Ast. mixed with 1/4 LD_{50} chlorophacinone. While, the synergistic

action of Astonin was clear with increase doses of chlorophacinone. The mortality percentage recorded 70% to mixture of 30 mg/kg b.wt. Ast. with LD_{50} chlorophacinone and reached to 100% when use dose of 90 mg/kg b.wt. Ast. mixed with the same dose of chlorophacinone compare with 50% mortality in treated rats by LD_{50} chlorophacinone alone. In fact Astonin-H resulted in migration of water form the primary urine back to blood with increase in the blood volume and the interstitial tissue fluid. The increase in the interstitial sodium and water causes an increase in tissue turgor (pressure suit) and a change in the electrical potentials on the outer cell membrane which will lead to increase vascular responsiveness to the circulating catecholamines. This action leads to increase in the blood pressure. These results is generally agree with those obtained by Gabr (1997) who studied the effect of some potentiators on the toxicity of warfarin to *R. rattus* collected from poultry farms that exhibited tolerance was studied. When warfarin bait was used only 60 % of animals were killed, but when calciferol, L¬histidine and sulphaduinoxaline were mixed with warfarin, the toxic effect of warfarin was increased to cause 100, 90 and 70% mortality, respectively. Also, he found that the effect of warfarin on bleeding time (B.T.) of *R. rattus* whereas B.T. values were considerably increased after treatment with 3.5 times than pre-treatment in the case of animals from Abou-Rawash area.

2. Effect on prothrombin time (P.T.):

Data in Table (4 & 5 and 6) showed that the P.T. increased 48h after treatment than 24h compared with pre-treatment for all treatments. There were significant differences between treatment after 24 and 48h. The highest value of P.T. were 899.67 and 890.33 second after 48h of treatment recorded at doses of LD_{50} + 90 and 100 mg/kg b.wt. Ast., respectively and the lowest value was (499.33 second) with LD_{50} chlorophacinone alone. Also, the Table (5) revealed that the highest value of P.T. was 153 second recorded at dose of $1/4LD_{50}$ + 100 mg/kg b.wt. Ast. after 48h of treatment, while the lowest value was (91.33 & 93.00 second) at $1/4LD_{50}$ chlorophacinone alone and 1/4 LD_{50} + 30mg/kg b.wt. Ast., respectively. But data in Table (6) showed that the highest value of P.T. was (62.33 & 60.67 second) recorded with the dose of 90 and 100 mg/kg b.wt. Ast. after 48h of treatment, respectively while the lowest values were (37.67 & 39.67 second) obtained with 10 and 30mg/kg b.wt. Ast., respectively.

These finding agreed with those obtained by Gabr (1997) reported that the effect of warfarin on prothrombin time (P.T.) of *R. rattus* was studied. Warfarin effect was obviously noticed in the case of animals from Abou-Rawash area whereas P.T. values were considerably increased after treatment with 3.6 times than pre-treatment. Kandil *et al.* (2008) observed that the effect of warfarin to *R. rattus* on (P.T.)

considerably differed from habitat to another. Warfarin enhanced PT values to 3.5, 3.2 and 2.6 time than pre- treatment for rat caught from houses, fields and poultry farm, respectively.

3. Effect on plasma total protein:

Data in Table (4 & 5 and 6) showed that the value of total protein post treatment increased after 48h than 24h compared with pre-treatment for all treatments. There were significant differences between treatments after 24 and 48h. The highest value of total protein after 48h of treatment was recorded with dose of LD_{50} + 100 mg/kg b.wt. Ast. (27.10g/L) and lowest value was (24.3g/L) obtained with the dose of LD_{50} chlorophacinone alone. Furthermore, data in Table (5) illustrated that the highest value of total protein after 48h of treatment was recorded with dose of $1/4LD_{50}$ + 100 mg/kg b.wt. Ast. (17.10g/L) and lowest value was (13.80g/L) obtained with the dose of 1/4LD50 chlorophacinone alone. Also, the data presented in Table (6) showed that the highest value of total protein was recorded (8.00g/L) with dose of 90 mg/kg b.wt. Ast. after 48h of treatment and lowest value was obtained with the dose of 10 mg/kg b.wt. Ast. (5.60g/L).

These results agree with the finding of Moussa (2005) found that 1/10, 1/30 and 1/90 LD_{50} of chlorophacinone, brodifacoum and warfarin increased the levels of plasma total protein of all treated animal groups of male albino rats and house mouse. Abd El-Bar (2007) illustrated that plasma level of total protein of animal group treated with brodifacoum and mixture of chlorophacinone and brodifacoum were significantly increased after one week of daily gastric administration.

4. Effect on plasma AST and ALT:

Data in Table (4 & 5 and 6) revealed that the AST increased post treatment of 48h than 24h compared with pre-treatment for all treatments. There were significant differences between treatment after 24 and 48h. The highest value of AST was recorded with the dose of LD_{50} + 100 mg/kg b.wt. Ast. (90.35 U/L) after 48h of treatment and the lowest value was (71.76 U/L) obtained with the dose of LD_{50} chlorophacinone alone. But, data presented in Table (5) indicated that the highest value of AST was recorded with the dose of $1/4LD_{50}$ + 100 mg/kg b.wt. Ast. (34.38 U/L) and the lowest value was (26.84 U/L) obtained with 1/4 LD_{50} + 30mg/kg b.wt. Ast. after 48h of treatment, respectively. As well as, data in Table (6) cleared that the highest value of AST was recorded with dose of 100 mg/kg b.wt. Ast. (10.30 U/L) and the lowest value was obtained with the dose of 10 mg/kg b.wt. Ast. (7.19 U/L) after 48h of treatment.

On the other hand, data in Table (4 & 5 and 6) showed that the value of ALT post treatment increased after 48h than 24h compared with pre-treatment for all

treatments. There were significant differences between treatment after 24 and 48h. The highest value of ALT was recorded with the dose of LD_{50} + 100 mg/kg b.wt. Ast. (31.12 U/L) and lowest value was (28.29 U/L) obtained with the dose of LD_{50} chlorophacinone alone after 48h of treatment. Also, Table (5) illustrated that highest value of ALT was recorded with dose of $1/4LD_{50}$ + 90 mg/kg b.wt. Ast. (10.05 U/L) and lowest value was (7.91 U/L) obtained with the dose of $1/4LD_{50}$ chlorophacinone alone after 48h of treatment. On the other hand, data presented in Table (6) indicated that the highest value of total protein was recorded (3.06 U/L) with dose of 100 mg/kg b.wt. Ast. and lowest value was obtained with the dose of 10 mg/kg b.wt. Ast. (2.0 U/L) after 48h of treatment.

The results of AST and ALT in generally agree with those obtained by Moussa (2005) found that 1/10, 1/30 and 1/90 LD_{50} of chlorophacinone, brodifacoum and warfarin increased the levels of plasma AST, ALT of all treated animal groups of male albino rats and house mouse. Abd El-Bar (2007) found that plasma ALT and AST level were significantly increased in animal groups treated with chlorophacinone, brodifacoum and mixture of them after one week of daily gastric administration compared to the control groups.

Table 1. Effect of the LD_{50} (5.4mg/Kgb.wt.) chlorophacinone alone or mixed with the different doses of Astonin®-H on the bleeding time (B.T.) and mortality percentage in albino rat, *Rattus norvegicus albinus*.

T.	Body weight (g)	B.T. (second)									M. %	Time to death (days)	
		Pre T.	Post treatment										
			12 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	Mean		Range	Mean
A	129	72	200	300	485	536	504	483	310	280	387	50	3-6	4.61
B	143.3	83	189	328	531	638	640	680	630	400	505	70	3-6	4.25
C	139.2	88	277	412	621	731	851	877	770	420	620	70	2-6	3.6
D	146.7	78	171	298	617	873	893	1224	891	711	710	90	4-5	4.6
E	143.3	89	264	384	655	804	1028	1268	-	-	734	100	2-5	3.5
F	145	83	270	370	700	912	1112	1273	-	-	773	100	2-5	3.17

T.: treatments; A:LD50 ; B:LD50+30 mg/Kg b.wt. Ast.; C:LD50+50 mg/Kg b.wt. Ast.; D:LD50+70 mg/Kg b.wt. Ast.; E:LD50+90 mg/Kg b.wt. Ast.; F:LD50+100 mg/Kg b.wt. Ast.; b.wt.: Body weight, Ast.: Astonin®-H drug.

Table 2. Effect of the $1/4LD_{50}$ (1.35mg/Kg b.wt.) chlorophacinone alone or mixed with the different doses of Astonin®-H on the bleeding time (B.T.) and mortality percentage in albino rat, *Rattus norvegicus albinus*.

T.	Body weight (g)	B.T. (second)									M. %	Time to death (days)	
		Pre T.	Post treatment										
			12 h	24 h	48 h	72 h	96 h	120 h	144 h	Mean		Range	Mean
A	130	85	105	195	297	340	211	172	153	210	0	-	-
B	137	65	113	215	285	351	205	191	167	218	0	-	-
C	125	82	109	233	315	392	287	203	185	246	0	-	-
D	120	74	127	265	420	473	350	265	210	301	0	-	-
E	120	101	125	208	334	506	564	388	294	346	0	-	-
F	124	94	166	274	488	554	606	330	287	386	20	8	8

T.: treatments; A: 1/4LD50; B: 1/4LD50+30mg/Kg b.wt. Ast.; C: 1/4LD50+50mg/Kg b.wt. Ast.; D: 1/4LD50+70 mg/Kg b.wt. Ast.; E: 1/4LD50+90 mg/Kg b.wt. Ast.; F: 1/4LD50+100 mg/Kg b.wt. Ast.; b.wt.: Body weight, Ast.: Astonin®-H drug.

Table 3. Effect of the different doses of Astonin®-H on the bleeding time (B.T.) and mortality percentage in albino rat, *Rattus norvegicus albinus*.

T.	Body weight (g)	B.T. (second)							M. %	Time to death (days)	
		Pre T.	Post treatment								
			12 h	24 h	48 h	72 h	96 h	120 h	Mean	Range	Mean
A	149.17	72	132	136	97	90	90	82	105	0	-	-
B	110.0	67	92	139	158	127	103	88	118	0	-	-
C	138.75	76	109	145	170	197	112	80	136	0	-	-
D	120.4	80	117	147	169	172	114	83	134	0	-	-
E	117.6	89	118	160	205	291	200	117	182	0	-	-
F	112.5	92	113	152	245	288	196	118	185	0	-	-

T.: treatments; A: 10mg/Kg b.wt. Ast.; B: 30mg/Kg b.wt. Ast.; C: 50mg/Kg b.wt. Ast.; D: 70mg/Kg b.wt. Ast.; E: 90mg/Kg b.wt. Ast.; F: 100mg/Kg b.wt. Ast.; b.wt.: Body weight, Ast.: Astonin®-H drug.

Table 4. Effect of the LD_{50} (5.4mg/Kg b.wt.) chlorophacinone alone or mixed with the different doses of Astonin®-H on some biochemical determination in albino rat, *Rattus norvegicus albinus*.

T.	Prothrombin time (second)			Plasma total protein (g/L)			AST (U/L)			ALT (U/L)		
	Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.	
		24h	48h		24h	48h		24h	48h		24h	48h
A	23.33	132.00^{d}	499.33^{e}	3.50	22.00^{d}	24.30^{d}	5.26	42.98^{ab}	71.76^{c}	2.06	10.74^{c}	28.29^{b}
B	25.67	185.00^{c}	695.67^{d}	3.30	22.20^{cd}	24.50^{d}	5.09	41.93^{b}	77.02^{b}	2.31	10.87^{c}	28.92^{b}
C	26.00	231.33^{b}	740.00^{c}	4.20	22.80^{bc}	25.20^{cd}	4.74	42.63^{ab}	80.18^{b}	1.94	11.68^{bc}	28.67^{b}
D	24.33	253.00^{a}	752.67^{b}	3.40	23.10^{ab}	25.90^{bc}	5.79	44.38^{ab}	87.72^{a}	2.19	12.13^{abc}	30.11^{a}
E	23.00	259.00^{a}	899.67^{a}	3.70	23.50^{a}	26.90^{ab}	4.56	43.16^{ab}	87.37^{a}	2.06	13.20^{a}	31.06^{a}
F	25.67	257.00^{a}	890.33^{a}	4.10	23.70^{a}	27.10^{a}	4.91	47.11^{a}	90.35^{a}	1.95	12.69^{ab}	31.12^{a}
L.S.D. (0.05)	N.S.	8.70^{***}	12.06^{***}	N.S.	0.68^{***}	1.07^{***}	N.S.	4.60	3.95^{***}	N.S.	1.44^{*}	1.19^{***}

T.: treatments; A: LD50; B:LD50+30 mg/Kg b.wt. Ast.; C:LD50+50 mg/Kg b.wt. Ast.; D:LD50+70 mg/Kg b.wt. Ast.; E:LD50+90 mg/Kg b.wt. Ast.; F:LD50+100 mg/Kg b.wt. Ast.; b.wt.: Body weight, Ast.: Astonin®-H drug; Mean values with the same letters in column are not significantly different at 0.05 level.

Table 5. Effect of the 1/4LD_{50} (1.35mg/Kg b.wt.) chlorophacinone alone or mixed with different doses of Astonin®-H on some biochemical determination in albino rat, *Rattus norvegicus albinus*.

T.	Prothrombin time (second)			Plasma total protein (g/L)			AST (U/L)			ALT (U/L)		
	Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.	
		24h	48h		24h	48h		24h	48h		24h	48h
A	19.67	59.00^{c}	91.33^{d}	4.30	9.90^{d}	13.80^{c}	5.26	17.02^{c}	28.07^{c}	2.06	4.01^{d}	7.91^{d}
B	18.67	60.00^{c}	93.00^{d}	3.90	10.10^{d}	15.00^{b}	5.44	17.72^{bc}	26.84^{c}	1.88	4.77^{cd}	8.29^{d}
C	19.33	62.00^{c}	102.00^{c}	3.40	10.70^{cd}	15.10^{b}	4.74	18.42^{bc}	29.30^{bc}	2.00	5.21^{c}	8.98^{c}
D	20.00	60.00^{c}	109.00^{b}	3.50	11.40^{bc}	16.20^{a}	5.79	20.00^{ab}	33.68^{a}	1.75	5.52^{bc}	9.36^{bc}
E	17.33	69.33^{b}	115.00^{b}	3.40	12.40^{ab}	16.90^{a}	4.56	22.46^{a}	32.28^{ab}	1.81	6.28^{ab}	10.05^{a}
F	21.33	84.00^{a}	153.00^{a}	4.20	13.10^{a}	17.10^{a}	5.97	22.28^{a}	34.38^{a}	1.75	6.34^{a}	9.99^{ab}
L.S.D. (0.05)	N.S.	3.93^{***}	6.00^{***}	N.S.	1.07^{***}	1.01^{***}	N.S.	2.57^{**}	3.83^{**}	N.S.	0.77^{**}	0.68^{***}

T.: treatment; A: 1/4LD50; B: 1/4LD50+30mg/Kg b.wt. Ast.; C: 1/4LD50+50mg/Kg b.wt. Ast.; D: 1/4LD50+70 mg/Kg b.wt. Ast.; E: 1/4LD50+90 mg/Kg b.wt. Ast.; F: 1/4LD50+100 mg/Kg b.wt. Ast.; b.wt.: Body weight; Ast.: Astonin®-H drug; Mean values with the same letters in column are not significantly different at 0.05 level.

Table 6. Effect of the different doses of Astonin®-H on some biochemical determination after oral administration in albino rat, *Rattus norvegicus albinus*.

T.	Prothrombin time (second)			Plasma total protein (g/L)			AST (U/L)			ALT (U/L)		
	Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.		Pre T.	Post T.	
		24h	48h		24h	48h		24h	48h		24h	48h
A	21.67	32.33^{d}	37.67^{c}	4.20	5.80^{b}	5.60^{c}	4.21	6.14^{b}	7.19^{c}	1.50	2.06^{b}	2.00^{c}
B	22.33	37.33^{c}	39.67^{c}	3.50	7.00^{a}	6.90^{b}	3.86	6.32^{b}	7.72^{bc}	1.56	2.25^{ab}	2.19^{bc}
C	19.67	41.67^{b}	46.33^{b}	3.30	6.90^{a}	7.10^{b}	3.86	7.54^{ab}	8.95^{ab}	1.43	2.50^{ab}	2.50^{abc}
D	18.67	40.67^{bc}	46.00^{b}	3.40	7.00^{a}	7.40^{ab}	4.39	7.89^{a}	9.12^{ab}	1.37	2.56^{ab}	2.62^{ab}
E	22.33	55.67^{a}	62.33^{a}	4.30	7.30^{a}	8.00^{a}	4.03	8.59^{a}	10.17^{a}	1.31	2.87^{a}	2.93^{a}
F	24.00	54.67^{a}	60.67^{a}	4.40	7.40^{a}	7.60^{ab}	4.21	8.42^{a}	10.3^{5a}	1.43	2.94^{a}	3.06^{a}
L.S.D. (0.05)	N.S.	3.56^{***}	3.68^{***}	N.S.	0.92^{*}	0.86^{**}	N.S.	1.43^{*}	1.46^{**}	N.S.	0.7	0.86^{**}

T.: treatment; A: 10mg/Kg b.wt. Ast.; B: 30mg/Kg b.wt. Ast.; C: 50mg/Kg b.wt. Ast.; D: 70mg/Kg b.wt. Ast.; E: 90mg/Kg b.wt. Ast.; F: 100mg/Kg b.wt. Ast.; b.wt.: Body weight, Ast.: Astonin®-H drug; Mean values with the same letters in column are not significantly different at 0.05 level.

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التأثير التنشيطي لمركب الاستونين–هـ على سمية مبيد الكلوروفاسينون ضد الفار الأبيض ***Rattus norvegicus albinus*** **(Berk)**

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تم استخدام مركب أستونين–هـ لرفع كفاءة مبيد القوارض المانع لتجلط الدم (الكلوروفاثينون) تحت الظروف المعملية. وأظهرت النتائج ما يلي:

- خلط الأستونين مع الجرعات المنخفضة من الكلوروفاثينون وهي ربع الجرعة النصفية المميتة تؤدي إلى حدوث زيادة في قياسات تجلط الدم ونسبة الموت ضد الجرذان البيضاء.
- تم تسجيل نسبة موت ٢٠٪ عند استخدام جرعة ١٠٠مجم/كجم أستونين–هـ من وزن الجسم مع ربع الجرعة النصفية المميتة من الكلوروفاثينون بينما لم يتم تسجيل أي نسبة موت عند استخدام الاربع جرعات الاولي وهي ٣٠ ، ٥٠ ، ٧٠ ، ٩٠ مجم/كجم أستونين–هـ من وزن الجسم مع نقس الجرعة من الكلوروفاثينون.
- وتم تسجيل اعلي نسبة موت ٩٠ ، ١٠٠٪ عند معاملة الفئران بجرعات ٧٠ ، ٩٠ مجم/كجم أستونين–هـ من وزن الجسم مخلوط مع مع الجرعة النصفية المميتة للكلوروفاثينون على التوالي مقارنة مع نسبة موت ٥٠٪ عند معاملتهم بالجرعة النصفية المميتة فقط وتم الموت خلال (٤–٥)، (٢–٥) ، (٣–٦) يوم لكل معاملة، على التوالي ، وزاد ايضا وقت النزف تدريجيا لكل معاملة على التوالي. وفي نفس الاتجاه زاد زمن البروثرومبين الي اكثر من الضعف عند خلط الاستونين– مع الكلوروفاثينون مقارنة مع الكلوروفاثينون منفردا.