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In Vitro Anthelmintic activity of some Novel N-substituted Imidazolin-5-one derivatives

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Abstract

A series of N-substituted imidazolin-5-ones were synthesized by oxazole condensation followed by amination with various heterocyclic amines in presence of ethanol and few drops of glacial acetic acid. Structures of the compounds were characterized on the basis of IR, C^{13}NMR and H^{1}NMR. Compounds 1a-7a and 1b-7b were screened for antihelmintic activity .Test results revealed that compound 5a and 5b showed paralysis time of 2.5 and 2.75 min and death time of 9 and 8.5 min while the standard drugs albendazole showed paralysis time of 11 min and death time of 21min, respectively, at the same concentration of 50mg/ml. All compounds were found to posses both vermifuge and vermicide properties.

Key-Words: N-substituted imidazolin-5-ones, antihelmintic activity, *Pheritima posthuma,* Albendazole

Introduction

Helminthes are recognized as a major problem to livestock's throughout tropics[1]. Helminth infections are one of the most prevalent diseases in developing and developed countries[2]. Globally, an estimated 2 billion people are infected by intestinal nematodes[3]. Most diseases caused by helminthes are of a chronic and debilitating in nature, they probably cause more morbidity and greater economic and social deprivation among humans and animals than any other single group of parasites.

Antihelmintic or antihelmintic are drugs that expel helminthes parasitic worms (helminthes) from the body, either by stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing). However they have shown the development of resistance to some broad spectrum antihelmintic (benzimidazoles, levamisole, and avermectins) and also some narrow spectrum wormers such as the salicylanilides (closantel). Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some antihelmintic drugs is now an enclosing area in the field of research[4].

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Literature survey of most recent studies on imidazole heterocyclic nuclei has proved it to be versatile heterocyclic nuclei having a myriad spectrum of pharmacological activities like antihelmintic, antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer and antiviral activities [5-14].

The aim of the present work is to investigate the antihelmintic activity of newly synthesized different N-substituted imidazolin-5-ones. Imidazolin-5-ones are very effective against various helminthes in decades. Moreover there are certain helminthes, which are found to be resistant to the major classes of antihelmintic via benzimidazoles, imidazothiazoles and macrocyclic lactones. Therefore a search of these novel N-substituted imidazolin-5-ones and its derivatives leads to the evaluation of prototype compound with antihelmintic activity.

Material and Methods

Materials and reagents were procured from commercial suppliers of sigma-Aldrich and were used without further purification. All chemicals used in the present study were either of A.R or G.R quality. Melting points were determined in open glass capillaries using an GallenKamp (MFB-600) melting point apparatus and were uncorrected. IR spectra (KBr discs) were recorded by Shimadzu FT-IR Spectrophotometer, Model No.8400S (Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker (400 MHz) and (100 MHz) spectrometer (chemical shifts in ppm) in $CDCl_3$ using TMS as internal standard. The progress of the reaction and purity of synthesized compounds was established by thin layer chromatography (Silica Gel G).

Drugs and Chemicals

Sodium chloride (SD FINE-CHEMLIMITED- B.NO-IOZA-SDFCL-D09Y/0308/607/21),Gum acacia (LOBA-V-0324/1), Normal saline, Distilled water and N, N-Dimethylformamide (LOBA- B.NO-L1O1571306), Albendazole and vehicle (1% v/v gum acacia in normal saline were used. All the prototypes were dissolved in minimum quantity of 1% v/v gum acacia and then the volume was adjusted to 10 ml with normal saline for making the concentration of 25mg/ml, 50mg/ml with DMF).

Standard Drug

Albendazole was taken as a reference standard and the concentration of the standard drugs were prepared in 1%v/v gum acacia in Normal saline to give 50mg/ml

Experimental Procedure

General procedure for the synthesis of novel N-substituted imidazolin-5-ones: (Scheme-1)

STEP-1: Synthesis of 4-benzylidene-2-phenyloxazol-5(4H)-one (1-7)

Oxazolin-5-ones were prepared by condensation of 0.01 moles of Hippuric acid with 0.02 moles of different types of aromatic aldehydes in presence of 0.075 moles of acetic acid and 0.025 moles of Sodium Acetate .To this 2ml of water was added and the reaction was proceeded in a microwave for 5minutes at 70 watts. The reaction mixture was cooled, precipitate separated out, it was filtered, dried, recrystallized from methanol and confirmed by TLC and melting point.

STEP2: Synthesis of 4-benzylidene-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one (1a-7a and 1b-7b)

N-Substituted imidazolin-5-ones synthesis was preceded by amination of equimoles (0.001moles) of step **1**with various amino heterocyclic's in presence of ethanol and few drops of Glacial acetic acid. The reaction mixture was heated, cooled; the product formed was filtered, dried, recrystallized from methanol and conformed by thin layer chromatography and melting point. The physical data of the compounds were represented in table1.

Antihelmintic Activity

Indian adult earthworms of the genus and species, *Pheritima posthuma* (family: megascolecidae), were used to study the antihelmintic activity. The earthworms were collected from the water logged areas of soils in Vijayawada, Andhra Pradesh, India were washed with normal saline to remove all the fecal matter and waste surrounding their body. The earth worms (*Pheritima posthuma*) 5-8 cm in length and 0.2-0.3 cm width weighing 0.8–3.04 g were used for all experiment protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the antihelmintic activity

Procedure

The earthworms of equal size and weight were divided into four groups of six earthworms in each group and washed thoroughly to remove mud and fecal matter [15]. The standard drug (albendazole) and test compounds were dissolved in minimum quantity of dimethyl sulphoxide and dimethyl formamide respectively adjusted the volume up to 10 ml with normal saline solution (control) to get the concentrations of 50 mg/ml. Six earthworms of nearly equal size were placed in Petri dishes containing standard drug solution and test compound's solutions of above mentioned concentrations at room temperature. The time taken in minutes for complete paralysis and death were recorded and then mean paralysis time and mean lethal time for each sample was calculated. The time taken for worms to become motionless was noted as paralysis time and time taken for worms when they do not respond to any external stimuli was taken as time for death. The mean paralysis time and death time of the earthworms for different test compounds and standard drug are tabulated in Table 5 and represented in fig-1

Statistical Analysis

Results were expressed as mean±s.e.m. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P < 0.05$.

Results and Discussion

Physicochemical and analytical data of the synthesized compounds1a-7a and 1b-7b including molecular formula, molecular weight, percentage yield, melting point, are shown in Table 1. Analytical data including IR, NMR spectral data is presented in Table 2, 3, 4 whereas results of antihelmintic studies are tabulated in Table 5. It was found in the antihelmintic studies of the titled compounds that compound 5a and 5b was found to be the most potent compound in this series which showed antihelmintic activity comparable to that of standard drug. Whereas compounds like 2a and 2b showed high activity, compounds,4a,4b,6a and 6b showed moderate antihelmintic activity and rest of other compounds 3a,3b,7a and 7b showed very less antihelmintic activity .the structure-activity relationship studies based on the above results clearly indicate that compounds with electron donating groups on the aromatic ring showed increased potency .the intense activity of the compounds is also greatly influenced by the amount of activation or deactivation and position of the groups on the ring. The hydroxyl substitution at ortho position (5a) has higher significant activity when compared to the hydroxyl at para position which clearly indicates that ortho substitution is responsible for increased activity. The results also indicate the rise in activity with the increase in the number nitrogen's in the heterocyclic ring.

Conclusion

A series of new N-substituted imidazolin-5-one derivatives prepared by a novel method and their ability to paralyse and cause death of Indian earthworms. Though the mechanisms underlying this process remain to be fully elucidated detailed mechanistic studies and lead optimization of these N-substituted imidazolin-5-one derivatives are under investigation. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an approach to develop new potent antihelmintic prototypes for further optimization and development to get new leads in the treatment of helminth infestations.

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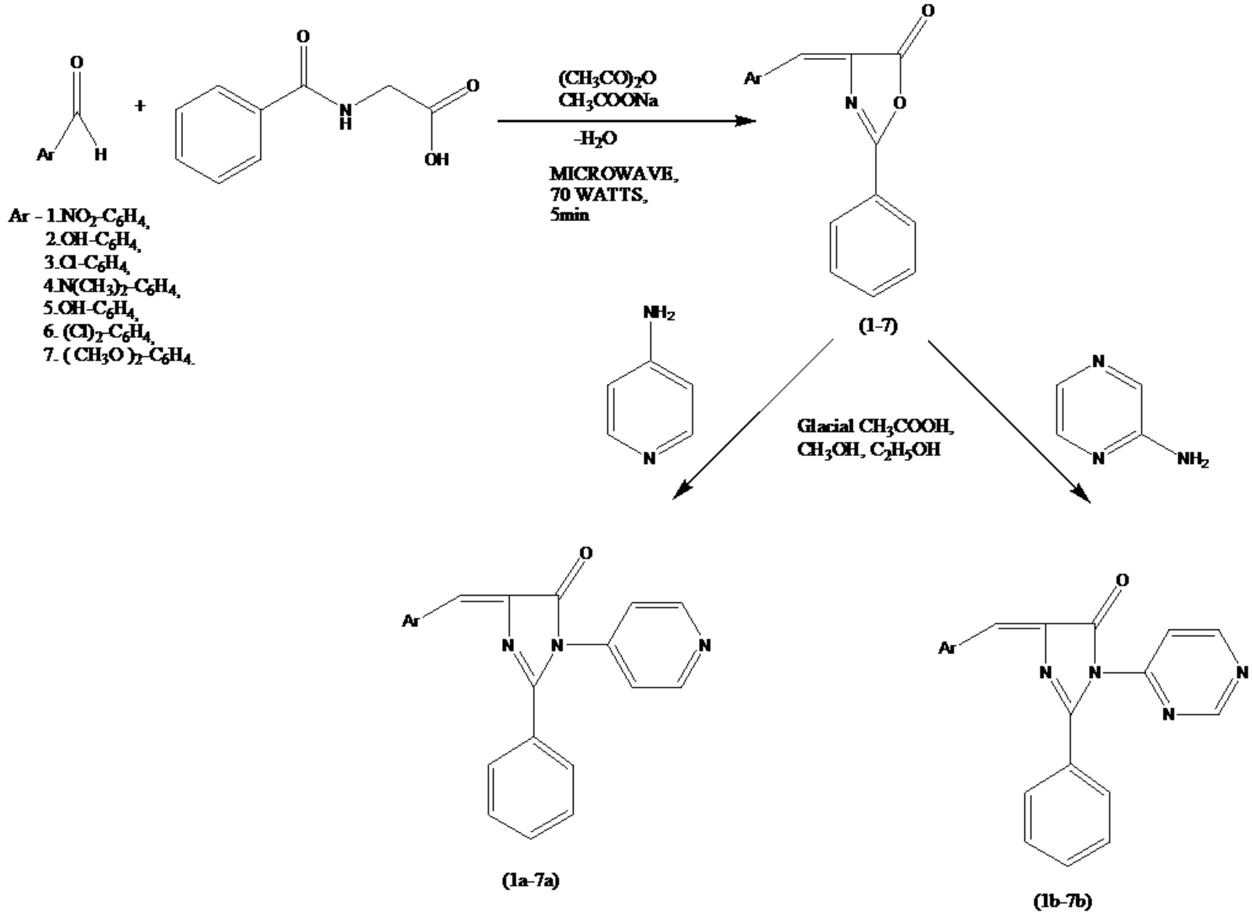

Scheme 1

Table 1: Physical data

Compound	IUPAC Name	MF	M W	Physical state		MP(0)C	% yield
				Colour	**State**		
1a	4-(4-nitrobenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{21}H_{14}O_3N_4$	370	White	Solid	226	63%
2a	4-(4-hydroxybenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{21}H_{15}O_2N_3$	341	Yellow	Solid	253	65%
3a	4-(4-chlorobenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{21}H_{14}ON_3Cl$	359.	Yellow	Solid	212	66%
4a	4-(4(dimethylamino)benzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{23}H_{20}ON_4$	368	Orange	Solid	211	60%
5a	4-(2-hydroxybenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{21}H_{15}O_2N_3$	341	Yellow	Solid	239	65%
6a	4-(2,4-dichlorobenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{21}H_{13}ON_3Cl_2$	394	Yellow	Solid	224	60%
7a	4-(3,4-dimethoxybenzylidene)-2-phenyl-1-(pyridin-4-yl)-1H-imidazol-5(4H)-one	$C_{23}H_{19}O_3N_3$	385	Yellow	Solid	196	61%
1b	4-(4-nitrobenzylidene)-2-phenyl-1-(pyrimidin-4-	$C_{20}H_{13}O_3N_5$	371	White	Solid	251	62%

	yl)-1H-imidazol-5(4H)-one						
2b	4-(4-hydroxybenzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{20}H_{14}O_2N_4$	342	Yellow	Solid	246	63%
3b	4-(4-chlorobenzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{20}H_{13}ON_4Cl$	358	Yellow	Solid	220	67%
4b	4-(4-(dimethylamino)benzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{22}H_{19}ON_5$	369	Orange	Solid	218	61%
5b	4-(2-hydroxybenzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{20}H_{14}O_2N_4$	342	Yellow	Solid	240	63%
6b	4-(2,4-dichlorobenzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{20}H_{12}ON_4Cl_2$	395	Yellow	Solid	227	61%
7b	4-(3,4-dimethoxybenzylidene)-2-phenyl-1-(pyrimidin-4-yl)-1H-imidazol-5(4H)-one	$C_{22}H_{18}O_3N_4$	386	Yellow	Solid	215	60%

Table 2: Characteristics IR absorption bands of different synthesized compounds are tabulated below

Compound	C-H	C=C	C=O	C=N	O-H	Ar-NO_2	C-Cl	C-O-C
1a	3036	1464	1646	510		1552		
2a	3033	1458	1747	1541	3274			
3a	3033	1458	1699	1540			775	
4a	3033	1458	1699	1517				
5a	3032	1457	1650	1419	3198			
6a	3099	1456	1644	1535			756	
7a	301062	1457	1646	15				1148
1b	3031	1460	1648	1513		1540		
2b	3032	1459	1698	1540	3202			
3b	3032	1458	1698	1540			727	
4b	3032	1457	1698	1519				
5b	3205	1456	1651	1522	3356			
6b	3065	1455	1646	1542			757	
7b	3059	1457	1699	1510				1138

Table 3: C^{13}NMR

Compound	Alkyl carbon(sp^3) (C-C)	Conjugated vinylic carbons (-C=C)	Aromatic carbons (Ar-C)	O=C-O	Ar-C=N	O=C-N	Ar-OH	Ar-NO_2
1a	-	115.42,113.94	113.40,114.24,115.23,117.16,127.91,123.96,121.74,132.54,130.61,155.02,157.02,164.09	162.09	151.02	-	-	70.8
2a	-	112.32,111.84	120.30,122.21,122.25,126.12,122.81,129.76,121.64,139.44,137.81,180.02,157.01,165.08	156.08	140.09	-	158.04	-
3a	-	111.52,118.94	121.20,123.34,124.23,126.26,137.91,122.76,123.64,131.54,138.61,160.02,158.09,166.08	161.08	157.04	-		
4a	40.03	111.82	127.81,128.78,132.28,133.32,134.85160.02,158.09,166.08	-	151.23	164.21	-	
5a	-	116.41,119.94	123.39,124.24,125.22,127.91,127.91,128.96,129.73,132.54,133.62,150.01,159.01,166.08	-	150.02	-	159.01	

6a	-	126.42,129.94	113.40,114.24,123.23,122.16,127.91,128.96,119.74,132.54,233.61,150.02,119.02,165.09	166.09	159.02	-		
7a	-	126.12,159.34	143.41,164.22,125.13,127.10,128.91,127.94,129.44,138.54,123.61,150.02,159.02,136.09	156.59	155.09	-		
1b	-	115.52,118.93	125.45,144.24,122.13,128.17,137.41,129.76,129.74,132.54,133.61,150.02,159.02,166.09	167.19	140.02	-	179.02	
2b	-	126.52,219.94	133.40,120.24,195.23,127.16,129.91,128.96,121.70,112.24,123.11,110.02,149.02,160.09	176.79	160.12	-	169.05	
3b	-	116.42,119.94	123.40,124.24,125.23,127.16,127.91,128.96,129.74,132.54,133.61,150.02,159.02,166.09	116.09	150.02	-	199.02	
4b	40.05	111.84	121.90,126.48,127.83,128.48,128.80,132.29,133.34,134.87,152.34,	-	152.34	166.34	-	
5b	-	116.41,119.94	123.40,124.24,125.23,127.91,128.52,128.96,129.74,132.54,133.61	-	150.01	166.08	159.01	
6b	-	115.22,109.94	103.40,114.24,125.23,107.16,117.91,118.96,119.74,132.54,123.61,110.02,159.02,166.09	160.12	169.05	-	197.02	
7b	40.04	111.05,111.82	125.31,126.45,127.12,127.80,128.66,128.78,132.00,132.27,133.33,134.85,	190.29	154.43	-	-	

Table4:H¹NMR

Compound	Hydrogen(n)	(ppm)	Multiplicity	Solvent
1a	a.Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.026,3.205 4.195,4.325 6.196-7.724 8.245-9.620	Triplet Singlet Multiplet Quintet	CDCl3
2a	a.Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.286,3.125 4.197,3.920 5.697-6.729 6.127-7.745	Triplet Doublet Multiplet Quintet	CDCl3
3a	a.Vinylic protons (-C**H**=C)(SP^2), b.Aromatic protons(Ar-**H**), c.**H**C=N	2.33,3.4 6.161-8.124 9.641,9.214	Singlet multiplet doublet	CDCl3
4a	a. Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.111 1.255,1.563 6.740-7.574	Singlet singlet multiplet	CDCl3

	e.3^{0}amine	8.137-8.159	quartet	
5a	a.Vinylic protons (-C**H**=C)(SP^2), b.Aromatic protons(Ar-**H**), c.**H**C=N	1.254,1.580 7.261-7.943 8.853,8.876	Singlet multiplet doublet	CDCl3
6a	a.Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.076,3.114 4.188,4.220 6.781-7.217 8.117-9.540	Doublet singlet multiplet quintet	CDCl3
7a	a.Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.094,3.154 4.298,4.450 6.635-7.907 8.125-9.745	Doublet singlet multiplet quintet	CDCl3
1b	a.Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.086,3.104 4.298,4.310 6.694-7.834 8.127-9.740	Doublet singlet multiplet quintet	CDCl3
2b	a. Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.671 1.665,1.651 7.840-8.644 9.37-8.159	Singlet singlet multiplet quartet	CDCl3
3b	a.Vinylic protons (-C**H**=C)(SP^2), b.Aromatic protons(Ar-**H**), c.**H**C=N d.**C-Cl**	1.254,1.580 7.261-7.943 8.853,8.876	Singlet multiplet doublet	CDCl3
4b	a. Alkylic protons (-C**H**$_3$)(SP^3), b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.109 1.255,1.563 6.739-7.573 8.132-8.158	Singlet singlet multiplet quartet	CDCl3
5b	a.Vinylic protons (-C**H**=C)(SP^2), b.Aromatic protons(Ar-**H**), c.**H**C=N	1.566 7.261-7.943 8.851-8.875	Singlet multiplet doublet	CDCl3
6b	a. Alkylic protons	3.209	Singlet	

	$(-CH_3)(SP^3)$, b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	1.275,1.663 6.739-7.573 8.102-9.158	singlet multiplet quartet	CDCl3
7b	a. Alkylic protons $(-CH_3)(SP^3)$, b.Vinylic protons (-C**H**=C)(SP^2), c.Aromatic protons(Ar-**H**), d.**H**C=N	3.534 1.955,1.963 6.739-7.573 8.112-9.298	Singlet singlet multiplet quartet	CDCl3

Table 5: Antihelmintic activity of novel N-substituted imidazolin-5-one derivatives

S No	Compounds	Dose	Paralysis time in minutes	Death time in minutes
			MEAN±S.E.M	MEAN±S.E.M
1	1a	50mg/ml	29±1.414	44±1.4
2	2a	50mg/ml	7.75±0.35	13±1.4
3	3a	50mg/ml	53±4.95	71.5±2.1
4	4a	50mg/ml	18±2.121	39±1.4
5	5a	50mg/ml	2.5±0.707	9±1.4
6	6a	50mg/ml	24.5±0.70	52±2.8
7	7a	50mg/ml	46±1.414	70±1.4
8	1b	50mg/ml	29.5±0.70	43±2.1
9	2b	50mg/ml	7.25±0.35	11.5±0.7
10	3b	50mg/ml	49±5.65	74±1.4
11	4b	50mg/ml	18.5±1.41	34±1.4
12	5b	50mg/ml	2.75±0.35	8.5±0.7
13	6b	50mg/ml	22±1.414	50±2.8
14	7b	50mg/ml	41.5±3.53	62±2.1
15	Control	Normal saline	-------	------
16	Albendazole	50mg/ml	11±1	21.5±2.1

Anova table

	SS	df	MS	F	R^2
Treatment	12256	3	4085	19.10	0.5242
Residual	11124	52	213.9		
Total	23380	55			

Each values is represented as mean ± standard error mean (n = 6)..Data are found to be significant by testing through one way ANOVA at 5 % level of significance ($p < 0.05$).

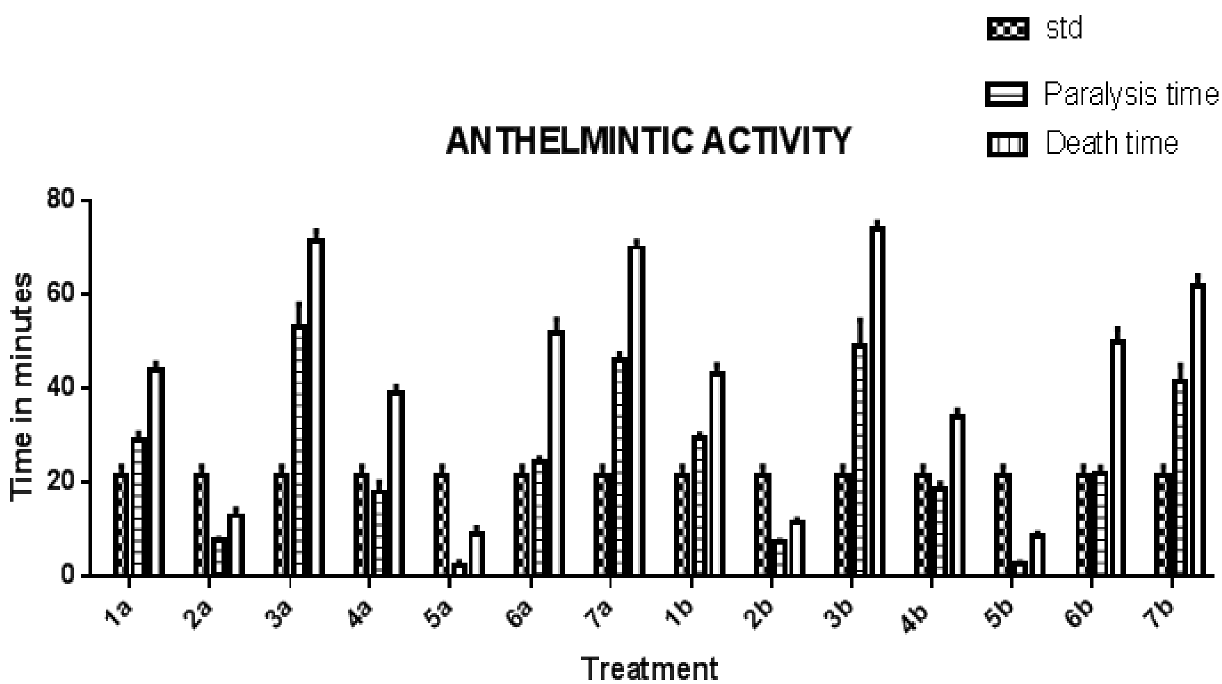

Fig. 1: Anthelmintic activity of compounds

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