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Original Article

Inhibition Kinetics of Acetylcholinesterase and Phosphatases by the Active Constituents of *Terminalia arjuna* and *Tamarindus indica* in the Cerebral Ganglion of *Lymnaea acuminata*

Neelam Soni, Dinesh Kumar Singh, Vinay Kumar Singh*

ABSTRACT

Introduction: Paper demonstrates effect of the active molluscicidal components arjunolic acid (*Terminalia arjuna* bark) and procynadine (*Tamarindus indica* seed) on the activity of acetylcholinesterase (AChE) and phosphatases (ACP/ALP) in the cerebral ganglion of snail *Lymnaea acuminata*. **Materials and Methods:** Kinetics of AChE/ACP/ALP inhibition in the cerebral ganglion of snail *Lymnaea acuminata* was studied via *in vivo* (40% and 80% of 96 h LC_{50}) and *in vitro* treatments (0.3 *µg* to 7.0 *µg*) of the column purified fractions, arjunolic acid and procynadine. **Results:** *In vivo* exposure of procynadine and arjunolic acid significantly inhibit acetylcholinesterase (AChE), acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in the cerebral ganglion of *L. acuminata* exposed to 80% of 96h LC_{50}. In *in vitro* treatment maximum inhibition in AChE/ACP/ALP activities in the cerebral ganglion of snail were noted when exposed to 7.0 *µg* of arjunolic acid and 0.9 *µg* of procynadine. Column purified fraction of *T. arjuna* bark and *T. indica* seed caused non-competitive and uncompetitive inhibition of AChE activity, respectively. Column purified fraction and arjunolic acid of *T. arjuna* bark caused uncompetitive inhibition of ACP while column purified fraction and procynadine of *T. indica* seed caused competitive inhibition. Competitive-non-competitive inhibition of ALP activity in the cerebral ganglion of *L. acuminata* was observed after treatment of column purified active components of both plants. **Conclusions:** The molluscicidal activity of *T. arjuna* bark (arjunolic acid) and *T. indica* seed (procynadine) against snail *L. acuminata* is due to the inhibition of AChE/ACP/ALP. Their inhibition kinetics against AChE/ACP/ALP, were different in cerebral ganglion of snail.

Key words: Enzymes, *Lymnaea acuminata*, *Terminalia arjuna*, *Tamarindus indica*, Arjunolic acid, Procynadine.

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INTRODUCTION

Fasciolosis is a serious infectious parasitic disease of domestic ruminants and human worldwide.[1,2] In tropical countries fasciolosis is frequently referred as the second most important parasitic disease after malaria and third most prevalent parasitic disease in the world in the terms of socio-economic and public health.[3] Trematode of genus *Fasciola*; namely *Fasciola hepatica* and *F. gigantica*[4,5] are the causative agent of fasciolosis.[6] About 250 million sheep and 300 million cattle are potentially affected by the fasciolosis world-wide.[7] Over the past three decade fasciolosis is recognized as an important emerging zoonotic disease of human and categorized as neglected tropical disease (NTD).[8] The incidence of human fasciolosis has also been reported in different state of India.[9] Life cycle of these trematodes involve snail *Lymnaea acuminata* as intermediate host.[10] One solution to tackle the problem of fasciolosis is to destroy the carrier snail, which is an essential link in the life cycle of liver flukes. The use of synthetic or plant molluscicides is the best method of snail control.[11,12] Synthetic molluscicides have created serious environmental hazards arising from contamination of ecosystem and its toxicity to non-target animals. Alternatively, molluscicides activity of plant origin are gaining more acceptance among farmers, as it is ecologically and culturally more acceptable than their synthetic counterpart.[13,14,15] It has been reported that plant of *Terminalia arjuna* and *Tamarindus indica* are the potent molluscicides against the snail *I. exustus*.[15]

The present work was aimed to study the inhibition kinetics of active molluscicidal components of both plants on AChE/ACP/ALP in the cerebral ganglion of the snail *Lymnaea acuminata* which will expose the mode of action of both plants in the snail body.

MATERIALS AND METHOD

Test materials

Fresh stem of *T. arjuna* bark (Voucher specimen no. # 3840) and *T. indica* (Voucher specimen no. 5648) seed were collected locally from Botanical garden of University campus. The specimens were identified and authenticated by Rtd. Prof. S. K. Singh, Taxonomist, Department of Botany, DDU Gorakhpur University, Gorakhpur, U.P. India.

Column purified fraction

50 ml ethanol extract of powder stem bark *T. arjuna* and seed of *T. indica* were subjected to silica gel

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(60-120) mesh (Qualigens Fine Chemicals, Mumbai, India) chromatography through 95×45 cm column. Five milliliters fractions of elutents were eluted with 95% ethanol for each column preparation. Ethanol was evaporated under vacuum and the remaining solids obtained from all 5 ml elutents were used for experimental analysis.

Active components

Arjunolic (2,3,23-Trihydrooxyolean-12-en, 28-oic acid) and Procynadine (cis,trans"-4,8"-Bi-(3,3',4',5,7-Pentahydroxyflavane) were parched from Sigma Chemical Co. U.S.A.

Test animal

Adult *Lymnaea acuminata* (2.35±0.30 cm in length) were collected locally from fresh water pond and pools of Gorakhpur, Uttar Pradesh, India. Snails were acclimatized for 72h in laboratory condition in dechlorinated tap water at 24±1°C.

Bioassay

In vivo treatments

Twenty experimental snails were kept in 3l of dechlorinated tap water. Each treatment 40% and 80% of 96h LC_{50} for 24h and 96h exposure of column purified fraction *T. arjuna* bark and *T. indica* seed and their active component arjunolic acid and procynadine were made, respectively (Table.1). Control group contained only 20 snails in equal volume of dechlorinated tap water without treatment. After 24h and 96h of the treatments snail were washed with water and the cerebral ganglion was quickly taken out for the measurement of various enzyme activity. Cerebral ganglion was removed and place in an ice cube. Afterwards, the cerebral ganglion was placed on filter paper to remove the adherent water and weight. Enzyme activity was performed in treated as well as in control group of test animal. In withdrawal experiments the snail were transferred from 24h exposure of 80% of 96h LC_{50} of different active components in to the freshwater then after 96h of enzyme assays were estimated (Table 1).

In vitro treatments

In *in vitro* treatments, column purified fraction and active molluscicidal component of *T. arjuna* bark and *T. indica* seed viz- arjunolic acid and procynadine, respectively were dissolve in ether and appropriate volume containing 1, 3, 5, and 7 µg of column purified fraction and active component arjunolic acid and 0.3, 0.5 0.7 and 0.9 µg of column purified fraction and active component procynadine were added to 10 mm path length cuvette. The ether was then allowed to evaporate. Each molluscicide was pre-incubated for 15 minute at 25°C with enzyme source and the enzyme activity was determined. The control treatment contains ether only.

Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were calculated by plotting Lineweaver-Burk plots for the hydrolysis of different concentration of substrate by the treated (5 µg) of *T. arjuna* bark (column purified fraction ((CPF) and arjunolic acid, and 0.7 µg of *T. indica* seed (column purified fraction (CPF) and procynadine) and untreated enzyme.

Acetylcholinesterase

Method of Ellman *et al*[16] was used for the measurement of AChE in the nervous tissue of treated snail. Cerebral ganglion (50 milligram) of *Lymnaea acuminata* was homogenized in 1.0 mL of 0.1M phosphate buffer pH 8.0 for 5 minutes in an ice bath then centrifuged at 1000g for 30 minutes at 4°C. The supernatant was taken as an enzyme source. Incubation mixture consisting of 0.1mL of enzyme source, 2.9 ml of 0.1M buffer pH 8.0, 0.1 ml of DTNB (5,5-dithio-bis-2-nitrobenzoic acid), and 0.02mL (ATChI) acetylthiocholine iodide, solution in distilled water was used in the measurement of AChE activity. The change in optical density of incubation mixture at 412nm was noted for 3 minutes after every 30 second at 25°C. Enzyme activity has been expressed as µmole "SH" hydrolyzed min/mg protein. For the estimation of kinetics constant (K_m) and maximum velocity (V_{max}) of AChE *in vitro* experiment 3.0×10^{-4}, 5.0×10^{-4}, 7.0×10^{-4}, 1.0×10^{-3}M of substrate acetylthiocholine iodide (ATChI) was used.

Acid Phosphatase

Acid phosphatase activity was determined by the method of Bergmeyer[17] as modified by Singh and Agarwal.[18] Two per cent (w/v) homogenates of cerebral ganglion of snail *L. acuminata* was made in ice cold 0.9% NaCl. Thereafter, homogenate was centrifuged at 5000rpm for 15 minutes at 4°C. 0.2 mL supernatant (enzyme source) was added to 1.0 mL of acid buffer subtract (prepared by dissolving 0.41g citric acid, 1.125g sodium citrate, and 165 mg 4-nitrophenyl phosphatase sodium salt were dissolved in 100mL of double distilled water) pre-incubated at 37°C for 10 minute. The incubation mixture was mixed thoroughly and incubated for 30 minute at 37°C. 4.0 mL of 0.1N NaOH was then added to the incubation mixture. The yellow color developed due to the formation of 4-nitrophenol. Optical density was estimated by spectrophotometer at 420nm. The activity of ACP is expressed as µ mole subtracts hydrolyzed/30 min/mg protein. Determination of kinetic constant (K_m) and maximum velocity (V_{max}) of acid phosphatase, *in vitro* was done by studying enzyme activity at different concentration (1.25×10^{-5}, 1.8×10^{-5}, 3.0×10^{-5}, and 5.4×10^{-5}M) of subtract p- nitrophenyl phosphate.

Alkaline phosphatase

Alkaline phosphatase activity in the cerebral ganglion of *Lymnaea acuminata* was measured by the method of Bergmeyer[17] as modified by Singh and Agarwal[18]. Tissue homogenate (2% w/v) was prepared in ice cold 0.9% NaCl and determined at 5000 g for 15 minute at 4°C. The supernatant was used as enzyme source 0.1 of enzyme 0.1mL of enzyme source was added to 1.0mLof alkaline buffer subtract (prepared by dissolving 375mg glycine, 10 mg $MgCl_2.6H_20$, 165mg 4-nitrophenol phosphate sodium salt in 42mL of 0.1N NaOH and a mixture was made up to 100mL with double distilled water) for 30 min at 37. Thoroughly mixed incubation mixture was incubated for 30 minute at 37°C. 10mL of 0.02N NaOH was then added to the incubation mixture. The yellow colour, developed due to the formation of 4-nitrophenol. The optical density was measured at 420nm. Activity of ALP is expressed as µ mole subtracts hydrolyzed/30 min/mg protein in the supernatant. Determination of kinetic constant (K_m) and maximum velocity (V_{max}) of alkaline phosphatase was done by studying enzyme activity at different concentration (1.25×10^{-5}, 1.8×10^{-5}, 3.0×10^{-5}, and 5.4×10^{-5}M) of subtract p- nitro phenyl phosphate.

Protein

Protein was estimated in the enzyme source supernatant by the method of Lowry *et al*.[19]

Statistical analysis

Each experimental was replicated at least six times and results were expressed as mean ± SE of Six replicates. Student's test was applied between control and treated groups to locate significant ($p< 0.05$) variations.[20]

RESULTS

In vivo enzyme inhibition

In vivo sublethal (40% and 80% of 96h LC_{50} for 24h and 96h exposure) treatment of *T. arjuna* and *T. indica* seed column purified fraction and its active component arjunolic acid and procynadine caused significant

(p< 0.05) inhibition in AChE, ACP and ALP activities in the cerebral ganglion of *Lymnaea acuminata* (Table 2 and 3). Maximum inhibition of AChE activity (52.84% of control) was observed in 80% of 96h LC_{50} of active component procynadine while in ACP activity maximum inhibition (34.66% of control) was observed in 80% of 96h LC_{50} of active component arjunolic acid. Maximum ALP inhibition (24.01% of control) was observed in 80% of 96 h LC_{50} of active component arjunolic acid in the cerebral ganglion of snail *L. acuminata*.

Significant (p< 0.05) recovery in AChE, ACP and ALP activity was observed in the cerebral ganglion of *L. acuminata* withdrawn from treatment. Maximum recovery in AChE (94.79% of control), ACP (82.13% of control) and ALP (89.65% of control) were noted in the cerebral ganglion

Table 1: Treatment with sublethal concentration of (40% and 80% of 96h LC_{50}) of active component and column purified fraction of *T. arjuna* bark and *T. indica* bark and seed against snail *L. acuminata*.

Treatment	Concentration of 96 h LC_{50}	40% of 96 h LC_{50}	80% of 96 h LC_{50}
T. arjuna bark CPF	3.21 mg/L	1.28 mg/L	2.56 mg/L
Arjunolic acid	1.30 mg/L	0.53 mg/L	1.06 mg/L
T. indica seed CPF	0.71 mg/L	0.28 mg/L	0.56 mg/L
Procynadine	0.31 mg/L	0.12 mg/L	0.24 mg/L

Table 2: *In vivo* effect of sublethal treatments of Column purified fraction and active components of *T. arjuna* bark and *T. indica* seed on AChE, ACP/ ALP activity in the cerebral ganglion of *L. acuminata*.

Enzyme activity	AChE (µ mole"SH" hydrolyzed/min/mg protein)				Withdrawal of 80% of 96h LC50 after 96h treatment
Control	0.668±0.008 (100)				0.653±0.008 (100)
Treatment	24h		96h		
	40% of 96h LC50	80% of 96h LC50	40% of 96h LC50	80% of 96h LC50	
T. arjuna bark CPF	0.546±0.001* (81.73)	0.469±0.003* (70.20)	0.453±0.003* (67.81)	0.382±0.009* (57.18)	0.619±0.004* (94.79)
Arjunolic acid	0.606±0.003* (90.71)	0.533±0.006* (86.52)	0.528±0.004* (79.04)	0.402±0.002* (60.17)	0.580±0.002* (88.82)
T. indica seed CPF	0.580±0.002* (86.82)	0.511±0.003* (76.49)	0.425±0.003* (63.62)	0.399±0.003* (59.73)	0.536±.007* (82.02)
Procynadine	0.474±0.002* (70.75)	0.416±0.002* (62.27)	0.416±0.003* (62.27)	0.351±0.002* (52.84)	0.567±.001* (86.83%)
Enzyme activity	ACP (µ mole substrate hydrolyzed/30min/mg protein)				
Control	20.22±0.05 (100)				19.59±0.03 (100)
Treatment	24h		96h		
	40% of 96h LC50	80% of 96h LC50	40% of 96h LC50	80% of 96h LC50	
T. arjuna bark CPF	15.61±0.06* (77.20)	12.68±0.03* (62.71)	9.20±0.04* (40.04)	7.59±0.02* (37.53)	16.09±0.01* (82.13)
Arjunolic acid	17.44±0.04* (76.25)	12.46±0.06* (61.62)	8.35±0.02* (41.29)	7.01±0.02* (34.66)	14.49±0.04* (73.76)
T. indica seed CPF	17.47±0.04* (86.31)	15.01±0.04* (74.23)	13.14±0.4* (64.98)	10.25±0.04* (50.69)	14.63±0.03* (74.68)
Procynadine	14.81±0.02* (73.29)	12.42±0.06* (61.42)	11.58±0.05* (57.27)	8.97±0.02* (44.36)	13.72±0.02* (70.03)
Enzyme activity	ALP (µ mole substrate hydrolyzed/30min/mg protein)				
Control	18.11±0.07 (100)				18.56±0.03 (100)
Treatment	24h		96h		
	40% of 96h LC50	80% of 96h LC50	40% of 96h LC50	80% of 96h LC50	
T. arjuna bark CPF	14.68±0.02* (81.66)	11.55±0.06* (63.73)	7.65±0.05* (42.24)	6.67±0.06* (36.83)	16.64±0.07* (89.65)
Arjunolic acid	12.93±0.04* (71.31)	10.07±0.04* (55.60)	5.54±0.03* (30.59)	4.35±0.03* (24.01)	14.13±0.01* (76.13)
T. indica seed CPF	15.74±0.03* (86.91)	10.21±0.04* (56.73)	11.80±0.02* (61.65)	9.32±0.07* (51.46)	15.12±0.04* (81.86)
Procynadine	13.52±0.09* (74.65)	11.60±0.04* (64.05)	10.18±.01* (56.21)	8.87±0.03* (48.97)	14.28±0.07* (76.93)

Values are mean ± SE of Six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (W/V) have been expressed as final concentration in aquarium water. CPF (column purified fraction). *- Significant (P<0.05) when student's t-test was applied between treated and control groups.

of snail withdrawn from 24h treatment of 80% of 96h LC_{50} of *T. arjuna* bark column purified fraction for next 96h of treatments (Table 1, 2).

In vitro enzyme inhibition

In vitro pre-incubation of 7µg of column purified fraction (67.00% of control) and arjunolic acid (69.75% of control) of *T. arjuna* bark significantly inhibits the AChE activity (Table 3). Treatment of 0.9µg of column purified fraction (66.39% of control) and procynadine (62.60% of control) of *T. indica* seed decreased the AChE activity respectively (Table 3). Pre incubation treatment of 7.0µg of column purified fraction and arjunolic acid of *T. arjuna* bark decreased the ACP activity to 46.08% and 39.13% of control, respectively (Table 3). Treatment of 0.9µg of column purified fraction (51.21% of control) and procynadine (45.16 % of control) of *T. indica* seed decreased the ACP activity respectively. *In vitro* treatment of 0.7µg of column purified fraction and active component (arjunolic acid) of *T. arjuna* bark decreased the ALP activity to 38.30% and 30.13% of control respectively (Table 3). Treatment of 0.9µg of column purified fraction and active component (procynadine) of *T. indica* seed decreased the ALP activity to 42.54% and 39.50 % of control, respectively.

Line weaver-Burk plot of column purified fraction and active components shows inhibited and uninhibited enzyme activity at different substrate concentration. The plot shows that K_m and V_{max} of uninhibited AChE (6.73×10^{-3}M and 0.94µ mole "SH" hydrolyzed/min/mg protein), ACP (1.42×10^{-5} M and 28.57 µ mole substrate hydrolyzed/30min/mg protein) ALP were, (2.02×10^{-5} M and 24.39 µ mole substrate hydrolyzed/30min/mg protein) respectively (Table 4; FigURE 1,2,3). K_m of column purified fraction and arjunolic acid of *T. arjuna* inhibited AChE activity were 6.73×10^{-3}M and 6.72×10^{-3}M, respectively. V_{max} of column purified fraction and arjunolic acid of *T. arjuna* inhibited AChE activity 0.57 and 0.60 µ mole "SH" hydrolyzed/min/mg protein respectively (Table 4; Figure 1 to 6). K_m and V_{max} value of inhibited AChE by column purified fraction and procynadine of *T. indica* seed were 5.94×10^{-3}M and 0.80µmole "SH" hydrolyzed/min/mg protein (Fig 1) and 6.35×10^{-3}M and 0.81µmole "SH" hydrolyzed/min/mg protein respectively (Fig 2). K_m and V_{max} value of inhibited ACP by column purified fraction and arjunolic acid 1.40×10^{-5}M and 25.00 µ mole substrate hydrolyzed/30min/mg protein and 1.40×10^{-5}M and 25.00 µ mole substrate hydrolyzed/30min/mg protein respectively, (Fig3). K_m and V_{max} value of inhibited ACP column purified fraction and procynadine of *T. indica* seed were 2.31×10^{-5}M and 28.57µmole substrate hydrolyzed/30min/mg protein, 2.48×10^{-5}M and 28.57 µmole substrate hydrolyzed/30min/mg protein respectively (Fig 4). Kinetics of inhibition of by column purified fraction and arjunolic acid show K_m and V_{max} value of inhibited ALP were 2.38×10^{-5}M and 22.22, 2.83×10^{-5}M and 22.22µmole substrate hydrolyzed/30min/mg protein respectively (Fig 5). K_m and V_{max} value of inhibited ALP column purified fraction and procynadine of *T. indica* seed were 2.27×10^{-5}M and 22.72µmole substrate hydrolyzed/30min/mg protein, 2.83×10^{-5}M and

Table 3: *In vitro* effect of sublethal treatments of column purified fraction and active components of *T. arjuna* bark and *T. indica* seed on AChE, ACP and ALP activity in the cerebral ganglion of *Lymnaea acuminata*.

Enzyme activity	AChE (µ mole"SH" hydrolyzed/min/mg protein)			
Control	0.668±0.008 (100)			
Treatment	1 µg	3 µg	5 µg	7µg
T. arjuna bark CPF	0.541±0.005 (89.12)	0.498±0.0003 (82.04)	0.469±0.0001 (77.26)	0.407±0.0004 (67.00)
Arjunolic acid	0.566±0.001 (93.24)	0.521±0.0002 (85.8)	0.464±0.001 (76.44)	0.421±0.001 (69.35)
Treatment	0.3 µg	0.5 µg	0.7 µg	0.9 µg
T. indica seed CPF	0.519±0.001 (85.50)	0.489±0.001 (80.56)	0.408±0.002 (67.21)	0.403±0.001 (66.39)
Procynadine	0.514±0.001 (84.67)	0.480±0.001 (79.01)	0.458±0.008 (75.54)	0.380±0.004 (62.60)
Enzyme activity	ACP (µ mole substrate hydrolyzed/30min/mg protein)			
Control	22.59±0.01 (100)			
Treatment	1 µg	3 µg	5 µg	7 µg
T. arjuna bark CPF	17.18±0.06 (78.12)	15.62±0.02 (69.14)	13.71±0.02 (60.69)	10.41±0.09 (46.08)
Arjunolic acid	16.77±0.02 (74.23)	14.64±0.02 (64.80)	12.35±0.01 (54.67)	8.84±0.04 (39.13)
Treatment	0.3 µg	0.5 µg	0.7 µg	0.9 µg
T. indica seed CPF	19.00±0.07 (84.10)	17.82±0.02 (78.88)	14.02±0.02 (62.06)	11.57±0.03 (51.21)
Procynadine	18.51±0.03 (82.97)	16.43±0.03 (72.73)	14.17±0.05 (62.62)	10.36±0.03 (45.86)
Enzyme activity	ALP (µ mole substrate hydrolyzed/30min/mg protein)			
Control	18.11±0.07 (100)			
Treatment	1 µg	3 µg	5 µg	7 µg
T. arjuna bark CPF	17.46±0.01 (78.12)	13.80±0.01 (61.60)	10.33±0.07 (46.11)	8.58±0.02 (38.30)
Arjunolic acid	17.05±0.04 (76.05)	11.77±0.03 (52.54)	9.67±0.01 (43.16)	6.75±0.01 (30.13)
Treatment	1 µg	3 µg	5 µg	7 µg
T. indica seed CPF	16.88±0.02 (75.55)	14.48±0.07 (64.64)	12.77±0.08 (57.00)	9.53±0.03 (42.54)
Procynadine	15.64±0.04 (69.82)	13.23±0.02 (59.06)	10.56±0.05 (47.14)	8.85±0.03 (39.50)

Values are mean ± SE of Six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (W/V) have been expressed as final concentration in the incubation mixture present in cuvette. CPF (column purified fraction).* Significant ($P<0.05$) when student's t-test was applied between treated and control groups.

Table 4: Kinetics constant (K_m and V_{max}) of *in vitro* enzyme inhibition by *T. arjuna* bark (CPF) and arjunolic acid (5.0 µg) and *T. indica* seed (CPF) and procynadine (0.7 µg) in snail *Lymnaea acuminata*

Treatments	Acetylcholinesterase		Acid phosphatase		Alkaline phosphatase	
	K_m (M)	V_{max}	K_m (M)	V_{max}	K_m (M)	V_{max}
Control	6.73×10-3	0.94	1.42×10-5	28.57	2.02×10-5	24.39
T. arjuna bark (CPF)	6.73×10-3	0.58	1.40×10-5	25.00	2.38×10-5	22.22
Arjunolic acid	6.72×10-3	0.60	1.40×10-5	25.00	2.83×10-5	22.22
T. indica seed (CPF)	5.94×10-3	0.80	2.31×10-5	28.57	2.27×10-5	22.72
Procynadine	6.35×10-3	0.81	2.48×10-5	28.57	2.83×10-5	23.25

Michaelis-Menten constant K_m and V_{max} of different enzyme were calculated from Lineweaver-burk plots (1/V versus1/S).

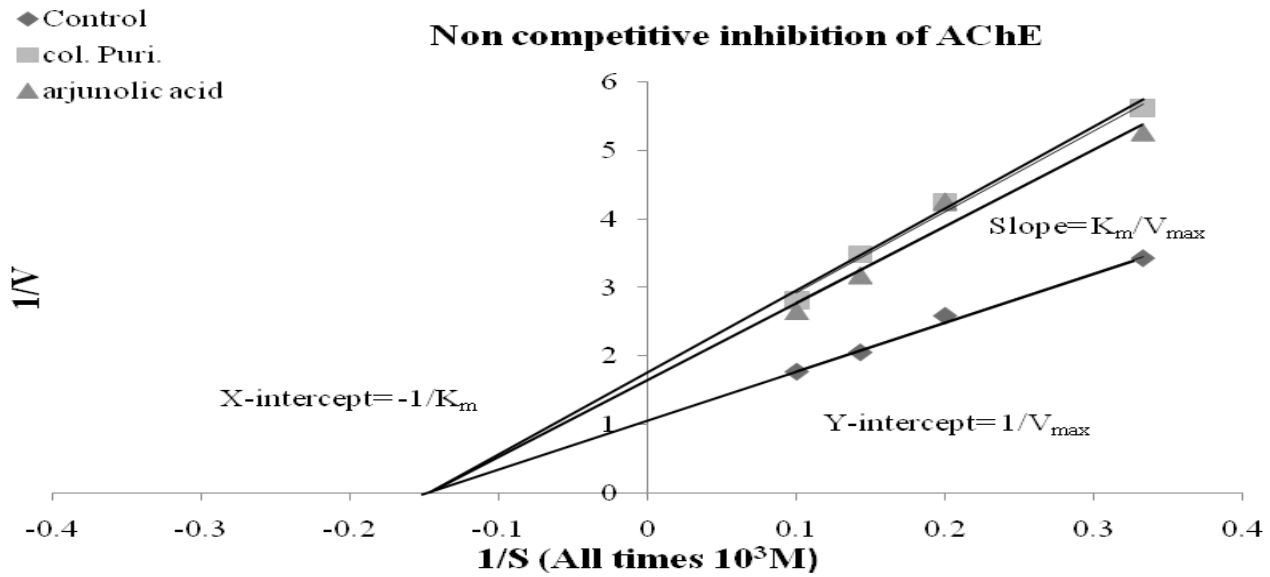

Figure 1: Line weaver-burk plots showing the effects of column purified fraction and active component arjunolic acid of *T. arjuna* bark (5.0 µg) on the inhibition of acetylcholinesterase (AChE) activity in the cerebral ganglion of snail *L. acuminata*.

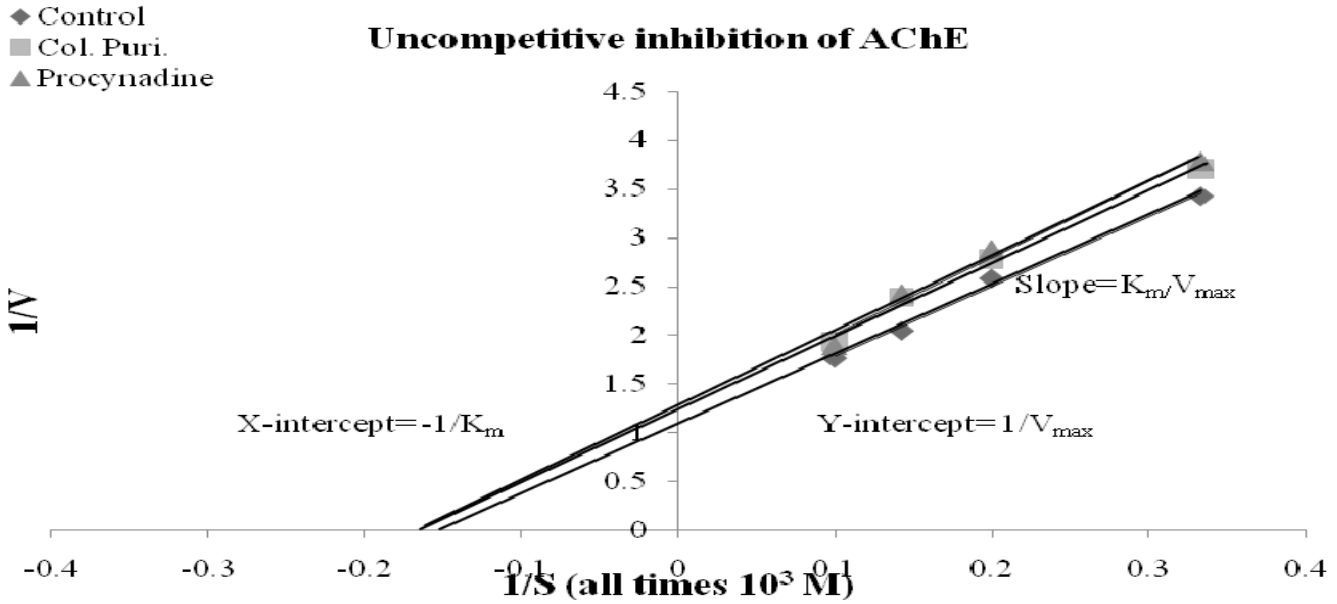

Figure 2: Lineweaver-burk plots showing the effects of column purified fraction and active component procynadine of T. indica seed (0.7 µg) on the inhibition acetylcholinesterase (AChE) activity in the cerebral ganglion of snail L. acuminata..

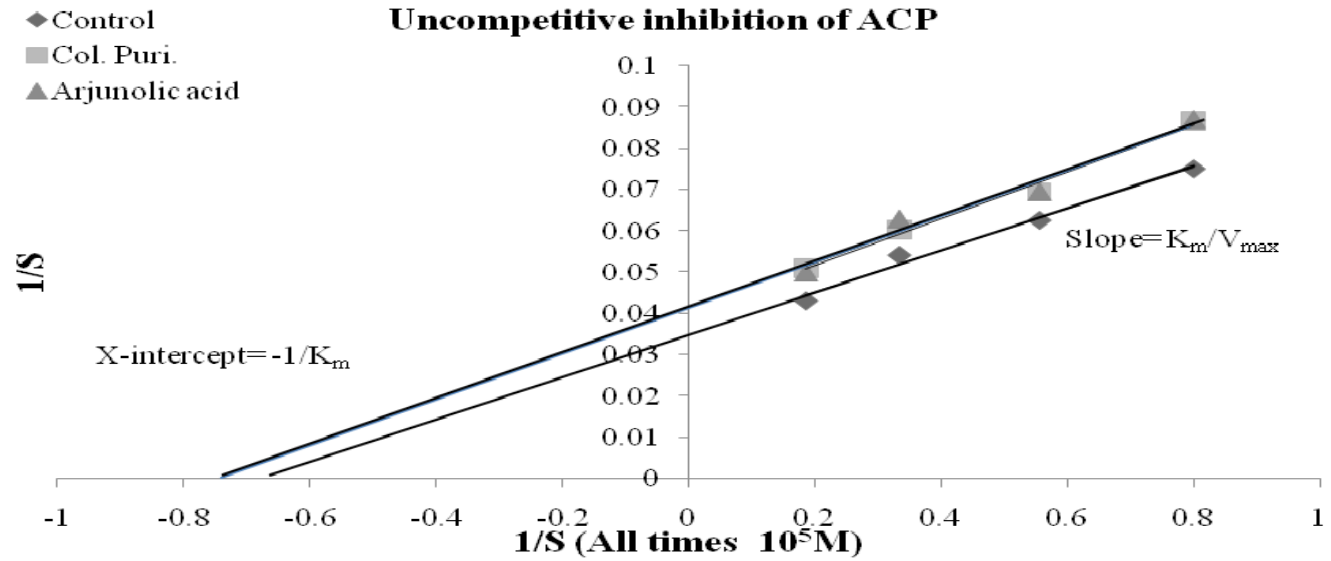

Figure 3: Lineweaver-burk plots showing the effects of column purified fraction and active component arjunolic acid of *T. arjuna* bark (5.0 µg) on the inhibition acid phosphatase (ACP) activity in the cerebral ganglion of snail *L. acuminata.*

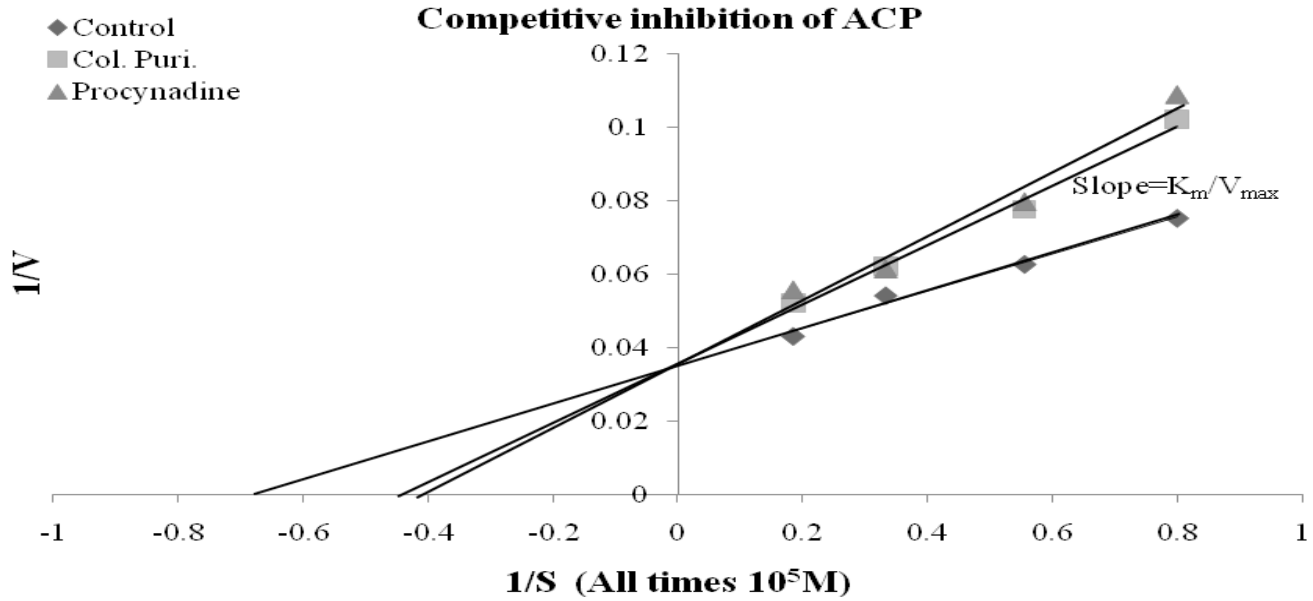

Figure 4: Lineweaver-burk plots showing the effects of column purified fraction (a) and active component procynadine (b) of *T. indica* seed (0.7 µg) on the inhibition acid phosphatase (ACP) activity in the cerebral ganglion of snail *L. acuminata.*

23.25 µ mole substrate hydrolyzed/30min/mg protein respectively (Fig 6).

DISCUSSION

Arjunolic acid and procynadine, active molluscicidal components of *Terminalia arjuna* bark and *Tamarindus indica* seed[13] caused significant inhibition of AChE, ACP and ALP activities in cerebral ganglion of *Lymnaea acuminata* in both *in vivo* and *in vitro* treatments. Maximum inhibition in AChE activity was noticed in cerebral ganglion of *L. acuminata* both in *in vivo* and *in vitro* treatment of procynadine. AChE is found in the synaptic membrane where it degrade the hydrolytic activity of the neurotransmitter acetylcholine, producing choline and acetate, a reaction important for the regulation of synaptic activity in central and peripheral neural system.[21] AChE plays a significant role in nerve conduction process at myoneutral junction of nerve ending of muscle tissue.[22] AChE inhibition caused accumulation of acetylcholine at the nerve synapses, so that the post synaptic membrane is in the state of permanent stimulation causing paralysis, ataxia, convulsion and death of animal.[23,24,35] *T. indica* seed extract caused dose dependent inhibition of phaspholipase A and other enzyme activity of snake venom.[25] Zhanq *et al*[26] reported the *in vitro* cytotoxic effect of procynadine on human hepatoma G2 cell.

Arjunolic acid of *T. arjuna caused* maximum inhibition of ACP and ALP enzyme activity in the cerebral ganglion of *L. acuminata*. Bark extract of *T. arjuna* have significant reduction in marker enzymes alkaline phosphatase (ALP), acid phosphatase (ACP), alanin amino transferase (ALT), aspartate amino transfrase (AST), and lactate dehydrogenase (LDH) in serum, tissue of liver and kidney in alloxan induced diabetic rats.[27] Arjunolic acid isolated from *T. arjuna* shows the cytotoxic activity against carcinoma and lymphoma cancer cell.[28] Lysozyme enzyme ACP plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis.[29,30] Pilo *et al*[31] reported the involvement of ALP play a

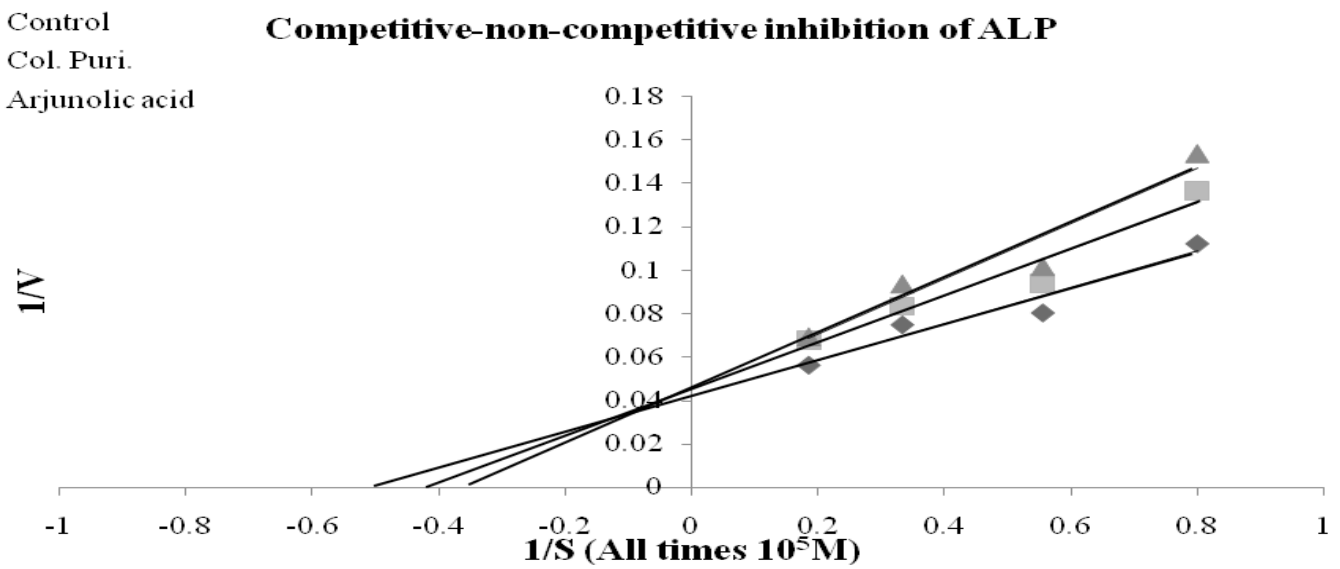

Figure 5: Lineweaver-burk plots showing the effects of column purified fraction and active component arjunolic acid of *T. arjuna* bark (5.0 µg) on the inhibition of alkaline phosphatase (ALP) activity in the cerebral ganglion of snail *L. acuminata*.

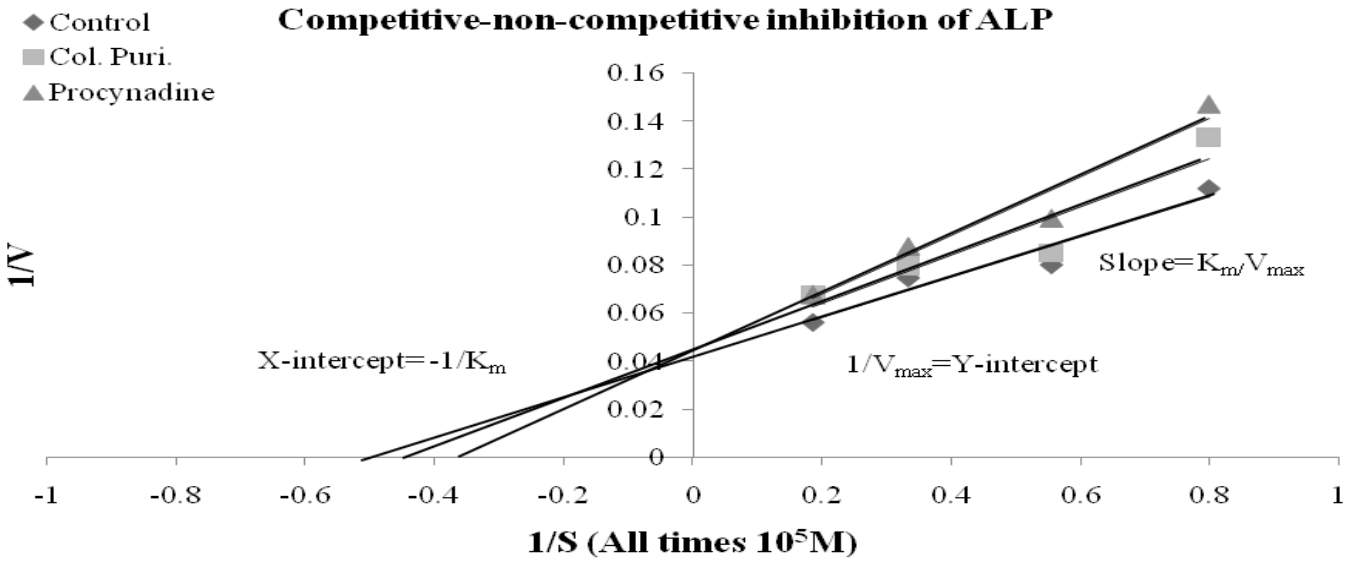

Figure 6: Lineweaver-burk plots showing the effects of column purified fraction and active component procynadine of *T. indica* seed (0.5 µg) on the inhibition of alkaline phosphatase (ALP) activity in the cerebral ganglion of snail *L. acuminata*.

critical role in protein synthesis, shell formation and other secretary activities in gastropods is noted by various workers.[31,32,33] Decrease in ALP activity may result altered transport and inhibitory effect on cell growth and multiplication, reduction in protein level and severe acidosis.[34]

Kinetics result shows same Km value of inhibited and uninhibited enzymes and different V_{max} on Lineweaver-Burk plots indicate that inhibition of AChE by column purified fraction and arjunolic acid of *T. arjuna* bark are non-competitive (Fig. 1). Slopes of inhibited and uninhibited AChE were not changed, both were parallel to each other, where as the intercept of inhibited and uninhibited AChE was changed that indicates column purified fraction and procynadine of *T. indica* seed caused uncompetitive inhibition in AChE activity (Fig.2). Slopes of inhibited and uninhibited ACP were not changed, both were parallel to each other, where as the intercept of inhibited and uninhibited ACP was changed that indicates column purified fraction and arjunolic acid of *T. arjuna* bark are non-competitive (Fig.3). Different Km value of inhibited and uninhibited enzymes and same V_{max} on Lineweaver-Burk plots indicate that inhibition of ACP by column purified fraction of *T. indica* seed and procynadine was competitive (Fig. 4). Column purified fraction of *T. arjuna* bark and arjunolic acid and column purified fraction of *T. indica* seed and procynadine were competitive-non-competitive inhibition of ALP as K_m and V_{max} of uninhibited and inhibited enzyme were different and slope of inhibited and uninhibited enzyme were changed, it is a mixed type of inhibition. In this situation the plots cross to the left of the 1/V axis but above the 1/S axis (Fig. 5, 6). It was reported that different plant derived molluscicide caused different mode of inhibition in key enzymes AChE, ACP and ACP activities in the cerebral ganglion of *L. acuminatea*.[35]

CONCLUSION

It can be concluded that alteration of AChE, ACP and ALP activity by *T. arjuna* bark and *T. indica* seed and their active constituents in the cerebral ganglion of snail *Lymnaea acuminata* may be responsible their molluscicidal activity.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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ABBREVIATIONS USED

AChE: Acetylcholineastrase; **ACP**: Acid phosphates; **ALP**: Alkaline phosphates; **CPF:** Column purified fraction.

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