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EFFECT OF CYCLOART-24-EN-3β-OL FROM *EUPHORBIA ROYLEANA* LATEX ON NEURO-ENZYME AChE AND OXIDATIVE METABOLISM OF FRESHWATER FISH, *CHANNA PUNCTATUS*

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Abstract

Cycloart-24-en-3β-ol isolated from *Euphorbia royleana* latex possesses potent piscicidal activity against freshwater predatory fish *Channa punctatus.* Their short as well as long-term exposure causes significant ($P<0.05$) time and dose-dependent reversible alteration in the oxidative metabolism of the fish *Channa punctatus* Cycloart-24-en-3β-ol also shifts fish respiratory pathway, inhibits energy production and AChE activity reversibly may be advantageous for their use as environmentally safe piscicide for controlling the predatory fish *C. punctatus* population from carp culture ponds.

Keywords: Cycloart-24-en-3β-ol, Triterpenoid, *Euphorbia royleana*, AChE, Oxidative metabolism, *Channa punctatus*

Introduction

Control of wild, resident, carnivorous species of fish e.g. *Channa punctatus, C. marulius, C. gachua*, *Wallago attu*, and other species from fish culture ponds prior to stocking is necessary because many species prey upon fingerlings of cultivated species of carps (Jhingran, 1991; Chiayvareesajja, et al., 1997). Air breathing predatory fish species cause special problems because they are carnivorous and survive in moist burrows even when the ponds are drained (Jhingran, 1991). Application of synthetic piscicides is one of the methods used to control predatory fish population (Gribgratok, 1981; Marking, 1992). Due to their long-term persistence, slow degradability in the water, toxicity to non-target organisms (Arasta et al., 1996) and accumulation inside the fish body, synthetic piscicides adversely affect the quality and the status of fish (Cullen and Connell, 1992) and contaminate the aquatic environment (Waliszewski et al., 1999). To solve this problem, studies have been carried out on the possibility of using local plants as piscicides (Chiayvareesajja et al., 1997; Singh and Singh, 2000), because the toxic effect of plant products is over within 7-12 days (Chakroff, 1976) and they are safe for users. A number of compounds (saponins, tannins, alkaloids, alkenylphenols, di- and tri-terpenoids etc.) present in several plants belonging to different families with piscicidal activities are used to control predatory fish (Tiwari and Singh, 2003; Tiwari and Singh, 2004).

Within the family Euphorbiaceae, the sixth largest among flowering plants, the genus *Euphorbia* L. alone accounts for almost a sixth of the whole group (Webster, 1994). The genus *Euphorbia* is composed essentially of latex bearing species (Lynn and Clevette-Radford, 1987). Many of them have been the objects of chemical and pharmacological investigation because of the irritant and medicinal properties of their latexes (Alberto Marco et al., 1997). These biological properties have been traced back in many cases to the presence of certain types of

diterpenes, most particularly phorbol derivatives, which have the tigliane skeleton and strong pharmacological effects (Evans, 1986).

Euphorbia royleana is a common medicinal plant of India and its latex in small doses is a purgative but in large doses it is acrid, counter-irritant and emetic and possesses cathartic and antihelimintic properties (Sastri, 1952). The medicinal as well as the poisonous properties of *E. royleana* latex are due to the presence of several compounds i.e. epitaraxerol, ellagic acid, euphol, taraxerol, sitosterol, m-hydroxy benzoic acid, 7-hydroxy3,4-benzcoumarin, 7-methoxy-3,4-benzcoumarin, 2',7-dihydroxy3,4-benzcoumarin etc. (Rastogi and Meharotra, 1993).

Piscicidal activities of several unidentified compounds isolated from *Euphorbia royleana* latex against predatory fish *C. punctatus* were well established and among them a compound isolated through methanol extraction was most effective, 24 hr LC_{50} 11.77 mg/L and 96 hr LC_{50} 9.19 mg/L (Tiwari and Singh, 2003).

Through the present investigation, we can now report the chemical characterization and structure elucidation of that compound isolated from *Euphorbia royleana* latex through methanol and its ultimate short as well as long-term biochemical effects on oxidative metabolism and acetylcholinesterase enzyme of fish, *Channa punctatus* (Bloch).

Materials and Methods

Collection of plant: The whole plants of *Euphorbia royleana* (Family-Euphorbiaceae) were collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur and identified by Prof. S.K. Singh at Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, U. P. (India), where a voucher specimen (number 2646) was deposited.

General Experimental Procedure: Melting points were determined on Sunvic apparatus. Silcia gel G (Merck) plates were developed with tolune and visualized under iodine vapours. Infrared spectra were recorded on Perkin Elmer-881 spectrophotometer (KBr), NMR spectra on Bruker DRX-300 with $CDCl_3$ as solvent. Mass spectra were carried out on a JEOL SX 102/DA-6000 Mass spectrometer. m-Nitobenzyl alcohol (NBA) was used as matrix.

Isolation and identification of compound from latex (MEx): In October, the white, milky latex of *Euphorbia royleana* was drained into glass tubes by cutting the stem apices. This latex was centrifuged at 1000xg for 20 mins for removing resin and then the resin free latex lyophilized at –40°C. The wet weight of one ml latex of *E. royleana* was 1.370 g and dry weight (lyophilized at –40°C) was 0.530 gm.

Dried, resin free, lyophilized powdered one-gram latex of *E. royleana* was dissolved in 200 ml methanol. After one-hr the whole solution was centrifuged at 5000xg for 25 mins at 35°C. After centrifugation the solution was passed through Whatman filter paper no. 1 (Whatman International Ltd., England). The supernatant was completely dried at 35°C temperature under reduced pressure. Thus a dried solid was obtained, which was thoroughly washed with methanol. The solvent was removed under reduced pressure and the soluble compound was re-crystallized to give white-yellowish crystals [(MEx) (540 mg; Rf 0.27)].

Collection and maintenance of fish: *Channa punctatus* (15.7±1.35 cm total length) were collected from Ramgarh lake of Gorakhpur district. The collected fish was stored in glass aquaria containing 100L de-chlorinated tap water. Experimental conditions of water were atmospheric temperature 30.5±1.5°C; water temperature 28±1°C; pH 7.3 to 7.5; dissolved oxygen 6.6 to 7.8 mg/L; free carbon dioxide 4.1 to 6.2 mg/L; bicarbonate alkalinity 106.0 to 109.0 mg/L (APHA/AWWA/WEF, 1998). Prior to experiment, fish were allowed to acclimate to laboratory conditions for one weak. Water was changed at every 24 hr.

Treatment conditions for biochemical estimation: Adult specimens of *C. puncatatus* were kept in glass aquaria containing 6L de-chlorinated tap water. Each aquarium stored ten experimental fishes. Fishes were exposed for 24 or 96hr exposure period to 40 and 80% of 24 or 96hr LC_{50} doses of MEx. After completion of treatment, the test fishes were removed from aquaria and washed with water, killed and liver and muscle tissues were quickly dissected out, freed from adipose and connective tissues, frozen in liquid nitrogen and stored at -70^0C, which was used for biochemical analysis. Control fishes were kept in same condition without any treatment.

Withdrawal experiment: In order to observe the effect of withdrawal of treatment of MEx, the fishes were exposed for 96h exposure period to 80% of the 96h LC_{50} and the one half of the animal was sacrificed and the activity of all above biochemical parameters were measured in liver and muscle tissue of fishes. The other half was transferred to

treatment free fresh water, which was changed every 24hr for the next seven days. After this, all the above biochemical parameters were again measured in liver and muscle tissue of fishes. Control fishes were kept in same condition without any treatment.

Each experiment was replicated six times and the values expressed as Mean±SE of six replicates. Student's 't' test was applied to express the level of significance between control and experimental data (Sokal and Rohlf, 1973).

Acid and alkaline phosphatase activity: was determined by the method of Andersch and Szcypinski (1947). Tissue homogenates (2% w/v) were prepared in ice-cold 0.9% sodium chloride solution and centrifuged at 5000xg at (0°C) for 15 min. Optical density was measured at 420 nm against a blank, prepared simultaneously. The enzyme activity was expressed as amount of ρ-Nitro phenol formed/30min/mg protein in supernatant. ρ- Nitro phenol was taken as standard.

Aspartate (AAT) and Alanine Amino transferase (ALAT) activity: were determined by the method of Reitman and Frankel (1957). Homogenate (50 mg/ml, w/v) was prepared in 0.25 M cold sucrose solution. Optical density was measured at 546 nm. The enzyme activity was expressed as μ moles of pyruvate formed/mg protein/hr.

Acetylcholinesterase (AChE) activity: was measured by the method of Ellman et al. (1961). Homogenate (50 mg/ml, w/v) was prepared in 0.1M-phosphate buffer (pH 8.0) for 5 min in an ice bath and centrifuged at 1000xg for 30min at 4^0C. The change in optical density at 412 nm, caused by the enzymatic reaction, was monitored for 3 min at 25^0C. Enzyme activity was expressed as μ mol 'SH' hydrolyzed/min/mg protein.

Lactic dehydrogenase (LDH) activity: was measured by the method of Sigma Diagnositics (1984). Homogenates (50 mg/ml, w/v) were prepared in 1 ml of 0.1 M phosphate buffer (pH 7.5) for 5 min in an ice bath. Enzyme activity was expressed as nanomoles of pyruvate reduced/min/mg protein.

Succinic dehydrogenase (SDH) activity: was measured by the method of Arrigoni and Singer (1962). Homogenate (50 mg/ml, w/v) was prepared in 1 ml of 0.5M potassium phosphate buffer (pH 7.6) for 5 min in an ice bath. Enzyme activity was expressed as μmoles dye reduced/min/mg protein.

Cytochrome oxidase (CyO) activity: was measurement by the method of Cooperstein and Lazarow (1951). Homogenates (50 mg/ml, w/v) were prepared in 1 ml of 0.33 M phosphate buffer (pH 7.4) for 5 min in ice bath. Enzyme activity was expressed as orbitrary units/min/mg of proteins.

Results

Identification of MEx compound: mp 100-101°C; Rf 0.27 (pure toluene); **IR ν_{max} (KBr)**: 3420.8, 3065.0, 2935.3, 1636.5, 1457.7, 1376.1, 1238.9, 1096.7, 1025.4, 887.7 and 714.0 cm^{-1}; **MS m/z** 426 [M^+] ($C_{30}H_{50}O$, 70.0%), 411 (100) [M^+ - methyl (15)], 393 (30.0) [M^+ - methyl – H_2O], 339 (5.0) [M^+ - side chain], 297 (6.0), 273 (4.0), 255 (3.0), 203 (25.0), 187 (20.0), 173 (20.0), 149 (35.5), 121 (60.0), 109 (72.0), 107 (62.0) and 105 (60.0%); **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 0.33 (1H,d, J=4.0 Hz, H-19), 0.54 (1H,d, J=4.0 Hz, H-19), 0.69 (3H,S, H_3-21), 0.75 (3H,S, H_3-18), 0.88 (3H,d, J=7.0 Hz, βH_3-28), 0.96 (3H,S,H_3-30), 0.99 (3H,S,αH_3-29), 1.60 (3H,S,H_3-26 or 27), 1.68 (3H,S,H_3-26 or 27), 3.26 (1H,m, αH-3), 5.09 (1H,t, J=5.0 Hz, H-24); **^{13}C-NMR** (300 MHz,$CDCl_3$): δ 13.9 (C-28), 15.5 (C-29), 17.6 (C-18), 18.3 (C-21), 18.9 (C-27), 20.1 (C-30), 21.5 (C-9), 24.4 (C-6), 24.7 (C-26), 25.4 (C-11), 26.0 (C-16), 26.4 (C-19), 28.0 (C-7), 29.2 (C-22), 29.7 (C-10), 30.8 (C-1), 31.3 (C-23), 32.8 (C-15), 34.9 (C-27), 35.2 (C-12), 36.1 (C-20), 45.2 (C-13), 47.0 (C-5), 47.9 (C-4), 49.6 (C-8), 50.9 (C-14), 52.2 (C-17), 78.8 (C-3), 105.0 (C-24) and 125.2 (C-25).

Biochemical study: Exposure to sub-lethal doses of MEx (4.71 mg/L, 9.14 mg/L for 24hr and 3.67 mg/L, 7.35 mg/L for 96 hr exposure period), caused significant ($P<0.05$) time and dose-dependent alterations in the activity of enzyme acid, alkaline phosphatase, ALAT, AAT, AChE, LDH, SDH and CyO in liver and muscle tissues of *Channa punctatus* (Tables 1 and 2).

Activity of acid phosphatase was inhibited to 40% and 48%, alkaline phosphatase was reduced to 56% and 66%, acetylcholinestersae enzyme activity was inhibited up to 40% and 38%, ALAT activity was increased to 190%

and 175%, AAT activity was increased to 179% and 160%; LDH activity was decreased to 60% and 55%, SDH activity was increased to 170% and 155% and CyO was decreased to 52% and 47% of controls after treatment with 7.35 mg/L of MEx for 96 hr in liver and muscle tissues of fish, respectively (Table 2). Seven days withdrawal experiment (Table 2) showed significant recovery in the activity of enzyme acid phosphatase, alkaline phosphatase, ALAT, AAT, AChE, LDH, SDH and CyO in both liver and muscle tissues of fish.

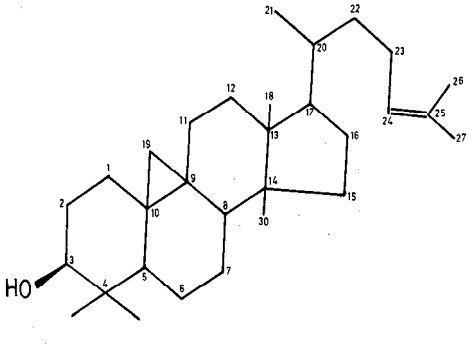

Figure 1(MEx): Cycloart – 24 – en - 3β – ol

Table 1. Changes in phosphatase, ALAT, AAT, AChE, LDH, SDH and CyO enzyme activity in different tissues of *C. punctatus* after exposure to 40% and 80% of LC_{50} (24h) of MEx for 24h exposure periods.

Parameter	Tissue	Control	40% of LC_{50} (4.71 mg/L)	80% of LC_{50} (9.14 mg/L)
AC	Liver	0.141±0.006 (100)	0.119±0.0020[a] (85)	0.085±0.001[a] (60)
	Muscle	0.134±0.004 (100)	0.089±0.0023[a] (66)	0.069±0.002[a] (52)
AL	Liver	0.489±0.007 (100)	0.440±0.003[a](90)	0.386±0.006[a] (79)
	Muscle	0.474±0.009 (100)	0.441±0.005[a] (93)	0.384±0.007[a] (81)
ALAT	Liver	3.66±0.005 (100)	4.76±0.05[a] (130)	5.86±0.07[a] (160)
	Muscle	2.90±0.003 (100)	3.48±0.04[a] (120)	4.21±0.06[a] (145)
AAT	Liver	1.43±0.003 (100)	1.72±0.07[a] (120)	2.17±0.03[a] (152)
	Muscle	1.26±0.015 (100)	1.41±0.06 (112)	1.75±0.04[a] (139)
AChE	Liver	0.190±0.001 (100)	0.162±0.006[a] (85)	0.081±0.004[a] (43)
	Muscle	0.240±0.004 (100)	0.166±0.003[a] (69)	0.125±0.009[a] (52)
LDH	Liver	414.12±0.20 (100)	331.30±0.23[a] (80)	289.88±0.25[a] (70)
	Muscle	390.10±0.16 (100)	327.68±0.24[a] (84)	288.67±0.21[a] (74)
SDH	Liver	52.33±0.12 (100)	65.41±0.15[a] (125)	72.74±0.27[a] (139)
	Muscle	50.21±0.20 (100)	60.25±0.22[a] (120)	67.78±0.21[a] (135)
CyO	Liver	40.17±0.17 (100)	32.14±0.24[a] (80)	28.12±0.24[a] (70)
	Muscle	38.20±0.18 (100)	28.27±0.20[a] (74)	24.07±0.23[a] (63)

Values are mean ± SE of six replicates; Values in parentheses are % change with control taken as 100%; Data were analyzed through student's test; [a]Significant ($P< 0.05$), when treated groups were compared with controls; Activity of enzyme AC/AL, ALAT/AAT, AChE, LDH, SDH and CyO expressed in ρ-Nitro phenol/30min/mg protein, μ moles pyruvate/mg protein/h, and μ mol 'SH'/min/mg protein, nanomoles of pyruvate reduced/min/mg protein, μ moles dye reduced/min/mg protein and orbitrary units/min/mg of proteins, respectively; AC: Acid phosphatase; AL: Alkaline phosphatase; ALAT: Alanine aminotransferase; AAT: Aspartate aminotransferase; AChE: Acetylcholinesterase; LDH: Lactic dehydrogenase; SDH: Succinic dehydrogenase; CyO: Cytochrome oxidase

Table 2: Changes in phosphatase, ALAT, AAT, AChE, LDH, SDH and CyO enzyme activity in different tissues of *C. punctatus* after exposure to 40% and 80% of LC_{50} (96h) of MEx for 96h exposure period and 7th days after withdrawal of treatment.

Parameter	Tissue	Control	40% of LC_{50} (3.67 mg/L)	80% of LC_{50} (7.35 mg/L)	7th after withdrawal (7.35 mg/L)
AC	Liver	0.141±0.006 (100)	0.098±0.002[a] (70)	0.056±0.003[a] (40)	0.127±0.003[b] (90)
	Muscle	0.134±0.004 (100)	0.071±0.003[a] (53)	0.064±0.005[a] (48)	0.107±0.001[b] (80)
AL	Liver	0.489±0.007 (100)	0.391±0.004[a] (80)	0.274±0.003[a] (56)	0.440±0.03[b] (90)
	Muscle	0.474±0.009 (100)	0.422±0.030[a] (89)	0.313±0.020[a] (66)	0.450±0.002[b] (95)
ALAT	Liver	3.66±0.005 (100)	6.15±0.003[a] (168)	6.95±0.13[a] (190)	4.40±0.05[b] (115)
	Muscle	2.90±0.003 (100)	4.44±0.12[a] (153)	5.08±0.10[a] (175)	3.25±0.03[b] (108)
AAT	Liver	1.43±0.003 (100)	2.15±0.03[a] (150)	2.49±0.04[a] (179)	1.64±0.041[b] (115)
	Muscle	1.26±0.015 (100)	1.76±0.02[a] (140)	2.02±0.05[a] (160)	1.32±0.03[b] (105)
AChE	Liver	0.190±0.001 (100)	0.119±0.004[a] (63)	0.076±0.003[a] (40)	0.180±0.001[b] (95)
	Muscle	0.240±0.004 (100)	0.145±0.004[a] (61)	0.090±0.004[a] (38)	0.221±0.002[b] (92)
LDH	Liver	414.12±0.20 (100)	310.59±0.50[a] (75)	248.47±0.34[a] (60)	409.98±0.55[b] (99)
	Muscle	390.10±0.16 (100)	265.27±0.47[a] (68)	214.56±0.41[a] (55)	374.50±0.63[b] (96)
SDH	Liver	52.33±0.12 (100)	69.60±0.33[a] (133)	88.96±0.35[a] (170)	55.99±0.44[b] (107)
	Muscle	50.21±0.20 (100)	61.26±0.42[a] (122)	77.83±0.38[a] (155)	52.72±0.77[b] (105)
CyO	Liver	40.17±0.17 (100)	25.71±0.52[a] (64)	20.89±0.49[a] (52)	38.96±0.34[b] (97)
	Muscle	38.20±0.18 (100)	21.01±0.30[a] (55)	17.95±0.23[a] (47)	36.29±0.53[b] (95)

- [b]Significant ($P< 0.05$), when withdrawal groups were compared with treated groups; other details are same as given in Table 1.

Discussion

MEx was isolated as crude solid from methanolic extract of *Euphorbia royleana* latex. Repeated crystallization of crude solid from MeOH afforded white-yellowish crystals (540 mg), mp 100-101˚C. It was identified as cycloart-24-en-3β-ol (Figure 1) by comparing its melting point, IR and mass spectra (Vijaya et al., 1982). However, its ^{1}H NMR, COSY and ^{13}C NMR spectral studies are being reported for the first time. Further, its occurrence in this plant is also being reported for the first time.

Exposure to MEx caused significant behavioural changes such as suffocation, body irritation, increased mucus secretion, loss of body equilibrium of fish (Tiwari and Singh, 2003). The nature and rapidity of the onset of these behavioural responses indicates that MEx is active at the neuromuscular system of the exposed fish *Channa punctatus.*

Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances (Bullock et al., 1977). From the result section, it is evident that compound MEx inhibit the activity of the enzyme acetylcholinesterase, present in synaptic regions which mediates transmission of impulses by breaking acetylcholine into acetic acid and choline (O'Brien, 1976). The acetylcholine at neural and neuro-motar regions upon accumulation causes 'hyper-excitability (Kabeer Ahammad Sahib and Ramana Rao, 1980), which in turn might also influence behavioural pattern and ultimately cause paralysis and loss of body equilibrium.

However, AChE inhibition is not the only factor contributing to abnormal behaviour patterns, since the behavioural patterns though mostly neurological, is also influenced by other metabolic changes (Koundinya, 1978).

In fact, there exists a close relationship between animal behaviour, co-ordination of brain, loss of body equilibrium, nervous function and metabolic and physiological state of an organism (Sambasiva Rao, 1999). No such behavioral symptoms and death occurred in control groups which indicating that no factor other than MEx was responsible for altered behavior and fish mortality.

Both the aspartate and alanine aminotransferases function as link between carbohydrate and protein metabolism by catalyzing the interconversion of strategic compounds like α-ketoglutarate and alanine to pyruvic acid and glutamic acid and aspartate and α-ketoglutaric acid to oxaloacetic acid and glutamic acid respectively (Martin et al., 1983). In present study, activity of both aminotranferases were higher in liver tissue than muscle tissue of control fishes which suggests that liver tissue is more efficient in utilizing amino acid for metabolic purposes.

By exposure of MEx, the activity of both the aminotransferases were highly elevated in both liver and muscle tissue, which confirms the augmentation of stress as a consequence of MEx. Since stress in general is known to elevate aminotransferase activities (Natarajan, 1985), such a situation takes place here in fish *C. punctatus* after exposure to MEx. It is likely that this stress might be the result of augmentation of energy demand during the toxicity period where the depletion in energy resources is high. To cope with this energy demands and to make up far the high decrease in tissue glycogen and glucose levels, the amino acids seem to take an active role to act as precursor of carbohydrate metabolism by being fed into TCA cycle through transamination reactions.

Vorbrodt (1959), has reported that phosphatase is an important enzyme of animal metabolism, which play an important role in the transport of metabolites across the membranes. Since, MEx have anti-phosphatase activity, so reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (Pilo et al.,, 1972).

Lactic dehydrogenase (LDH) forms the centre for a delicately balanced equilibrium between catabolism and anabolism of carbohydrates (Everse and Kaplan, 1973). LDH mediates inter-converson of lactate to pyruvate depending on the availability of NAD, Co-enzyme (Sambasiva Rao, 1999). The decrease in lactate activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis (Sambasiva Rao, 1999).

The higher activity of succinic dehydrogenase (SDH) in liver than muscle is due to higher distribution of mitochondria, since SDH is mitochondrially localized enzyme (Harper et al., 1979). SDH is active regulatory enzyme of the tricarboxlic acid cycles. The toxicity of MEx decreased the ability of fish to respire there by decreasing oxygen uptake. At the same time, the requirements of energy are geared up by starting the mobilization of the carbohydrate reserves for production of energy. Stress produced by toxicant had adverse effect on energy metabolism and caused the SDH activity to rise, it seems that it is a compensatory effect (Singh and Srivastava, 1999). Similar result was observed in snail *Lymnea acuminata* (Singh and Agarwal, 1993) and in rat (Gupta and Kapoor, 1975).

Inhibition of cytochrome oxidase suggests that MEx has a profound impact on the oxidative metabolism, possibly due to their influence on respiratory process like electron transport system (ETS). Decrease in cytochrome oxidase might be the result of reduced availability of O_2, which in turns reduced the capacity of ETS to produce ATP molecules (Sambasiva Rao, 1999). Compounds having anti-acetylcholinesterase activity are known to usually inhibit mitochondrial reactions like the function of the cytochrome oxidase in ETS (Stevens et al., 1972). Because MEx also has anti-AChE activity and affects Kreb's cycle, it diminishing the rate of ETS and oxidative phosphorylation, resulting in reduced synthesis of ATP.

Therefore due to a combination of the decrease of respiratory rates, the activity of oxidative enzymes and accumulation of lactic acid, coupled with the inhibition of LDH, SDH activity, the tendency towardsof shifts in the metabolism of carbohydrate more towards anaerobic metabolism is suggested..

Thus the isolated compound MEx, identified as cycloart-24-en-3β-ol, has potent piscicidal activity against the predatory fish *Channa punctatus* and its sub-lethal doses altered behavioural pattern, inhibited oxidative metabolism and suppressed energy production of fish. However, this was advantageous because the toxic action of this compound was reversible, despite their high toxicity. So, cycloart-24-en-3β-ol is a potential piscicide for controlling predatory fish *Channa puctatus* population from aquaculture ponds.

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