Toxic effects of taraxerol extracted from *Codiaeum variegatum* stem-bark on target vector snail *Lymnaea acuminata* and non-target fish

Toxicidad del taraxerol extraído del tallo de *Codiaeum variegatum* sobre el caracol *Lymnaea acuminata* y sobre peces

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ABSTRACT

The active compound taraxerol (triterpene) extracted from the stem-bark of *Codiaeum variegatum* Blume (Euphorbiaceae) was administered for 24h or 96h to the freshwater snail *Lymnaea (Radix) acuminata* Lamarck in order to test its lethality. It was observed that the molluscicidal activity of taraxerol was time as well as dose dependent for the snail at all the exposure periods. There was a significant negative correlation between LC values of taraxerol and exposure period, thus LC_{50} values decrease from 1.69 mg/L (24h) to 0.74 mg/L (96h) against *Lymnaea* (Radix) *acuminata* Lamarck. Toxicity experiments were also carried out on non-target freshwater fish *Channa punctatus* (Bloch) (Channidae, Ophicephalidae), which shares the habitat with snails, for environmental toxicity, if any.

Exposure of sub-lethal (40% and 80% of LC_{50}) doses of taraxerol over 24h caused significant alterations in carbohydrates and nitrogenous metabolism in nervous, hepatopancreas and ovotestis tissues of snail *Lymnaea acuminata*. Fish *Channa punctatus* also exposed to sub-lethal doses of taraxerol (40% and 80% of LC_{50} 24h of *Lymnaea acuminata*) for 96h periods in order to measure potential effects on non-target organisms , also show significant alteration in carbohydrates and nitrogenous metabolism in muscle, liver and gonadal tissues. This study also shows that there is partial recovery in these parameters in both snail and fish after the 7 day of withdrawal of the treatment, which supports the view that it is safe to use plant products as molluscicides for controlling snails in aquatic bodies.

RESUMEN

Se administró el principio activo taraxerol (un triterpeno) obtenido de los tallos de *Codiaeum variegatum* Blume (Euphorbiaceae) al caracol *Lymnaea (Radix) acuminata* Lamarck durante 24 o 96 horas, para probar su letalidad. La actividad molusquicida del compuesto depende tanto del tiempo como de la dosis para ambos periodos de exposición. Hay una correlación negativa significativa entre los valores LC del compuesto y el periodo de exposición, los valores LC_{50} descienden de 1,69 mg/L (24h) a 0,74 mg/L (96h) para los caracoles. También se probó el compuesto en el pez *Channa punctatus* (Bloch) (Channidae, Ophicephalidae), que comporte hábitat con el molusco, para determinar la posible toxicidad ambiental.

La exposición durante 24 horas a dosis subletales (40 y 80% de LC_{50}) provocó alteraciones en el metabolismo de carbohidratos y compuestos nitrogenados en tejidos

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nervioso, del hepatopáncreas y ovotestis del caracol. Exposiciones de 96 horas de los peces a las mismas dosis mostraron los mismos efectos sobre los tejidos muscular, hepático y gonadal. El estudio muestra que hay una recuperación parcial de los parámetros en ambos organismos al septimo día tras abandonar el tratamiento, lo que apoya la idea de que es seguro el uso de productos vegetales como molusquicidas en medios acuáticos.

KEY WORDS: *Lymnaea acuminata*, *Channa punctatus*, molluscicides, taraxerol, metabolism.
PALABRAS CLAVE: *Lymnaea acuminata*, *Channa punctatus*, molusquicida, taraxerol, metabolismo.

INTRODUCTION

Once a pesticide is released into the environment, chemical, physical, biological and other allied factors determine its fate and distribution in the ecosystem. Heavy use of pesticides to control pests results in the pollution of the environment affecting both target species snails, slugs etc. and many non-target species viz. Phytoplankton, Zooplankton, fish etc in freshwater ecosystems (GOPAL, KHANNA, ANAND AND GUPTA, 1981). Pesticides interfere with the physiological and metabolic function in animals, which sometimes result in death (ARASTA, BAIS AND THAKUR, 1996).

With growing awareness of environmental pollution caused by synthetic molluscicides (RITCHIE, 1973; SRIVASTAVA AND SINGH, 2001), efforts are being made to find molluscicides of plant origin. Being the product of biosynthesis, they are highly toxic and easily biodegradable in nature (MARSTON AND HOSTETTMAN 1987; SINGH, SINGH, MISHRA AND AGARWAL, 1996). Two diseases - schistosomiasis and fascioliasis - carried by aquatic snails, cause immense harm to man and his domestic animals (BALI, SINGH AND SHARMA, 1986; AGARWAL AND SINGH, 1988). Large populations of aquatic snails inhabiting freshwater bodies cause serious destruction of freshwater vegetation which ultimately affect the growth of organisms feeding on them (REINERT, 1972).

Fascioliasis caused by *Fasciola hepatica*, the large liver-fluke, is common in sheep, cattle, goats and other herbivorous animals throughout the world. The freshwater snail *L. acuminata* is the intermediate host of *Fasciola hepatica* and *Fasciola gigantica* (AGARWAL AND SINGH, 1988; SINGH AND AGARWAL 1992; YADAV AND SINGH 2001, 2002), which cause great harm to domestic animals. SINGH AND AGARWAL (1981) reported that 94% of buffaloes slaughtered in Gorakhpur of Uttar Pradesh, India were infected by liver fluke *Fasciola gigantica*.

The aim of the present study is to report on the molluscicidal activity of taraxerol extracted from stem-bark of *C. variegatum* , against the harmful snail *L. acuminata*. Its effects on biochemical parameters of target snail *L. acuminata* and non-target fish *Channa punctatus* were also studied. Freshwater fish *Channa punctatus* is a common fish in India and shares the habitat with the snails.

MATERIALS AND METHODS

The stem-bark of *C. variegatum* was collected locally from the Botanical garden of D.D.U Gorakhpur University Gorakhpur, where a voucher specimen is deposited and identified by Prof. S.K. Singh (taxonomist), Department of Botany, D.D.U, Gorakhpur University, Gorakhpur (U.P) India.

Extraction of active compounds from stem-bark: Pure taraxerol was isolated from the stem-bark of *C. variegatum* by the method of CHATTERJEE AND BANERJEE (1977). The stem-bark of *C. variegatum* was dried in an incubator at 37°C and dried stem-bark was powdered with the help of a mechanical device. The dried powdered stem-bark (2 kg) of *C. variegatum* was extracted in Soxhlet

apparatus with petrol, for about 70 hours and a little amount of concentrated solution was obtained. After evaporation of the solvent by vacuum pump, the isolated compound in dried form was obtained. The organic constituents present in stem-bark i.e. taraxerone-2, taraxerol, taraxeryl acetate-4 and sitosterol were extracted with petrol. Taraxerol is soluble in organic solvents such as $CHCl_3$ and $CHCl_3$-MeOH. Identification of the isolated compound was further confirmed with an authentic sample of taraxerol ($C_{32}H_{48}O_9$), supplied by Sigma chemical Co. U.S.A.

The extracted compound was stored in an airtight desicator and used for toxicity experiments. Toxicity experiments were performed by the method of SINGH AND AGARWAL (1988). The freshwater snail *L. acuminata* were exposed to four different concentrations of taraxerol, 0.7 mg/L, 1.0 mg/L, 1.4 mg/L and 1.7 mg/L respectively for 24h, 48h, 72h and 96h exposure period. Mortality was recorded every 24h up to 96h. Ten snails were kept in 3L de-chlorinated tap water. Control animals were kept in similar conditions without any treatment. Each set of experiments was replicated six times. Effective doses (LC values), upper and lower confidence limits, slope value,'t'ratio, 'g' factor and heterogeneity were calculated by Probit log analysis method using POLO computer programme of RUSSELL, ROBERTSON AND SEVIN (1977).

For environmental toxicity, if any, the toxic effect of taraxerol was also studied in mixed populations of target organism snails and non-target organism fish. In this experiment, groups of 10 snail *L. acuminata* and 10 fish *C. punctatus* were put together in 6L de-chlorinated tap water, which were exposed to 2.92 mg/L (LC_{90} 24h of *L. acuminata*) of taraxerol for 24h.

Treatment protocol for Dose-Response relationship: *L. acuminata* was kept in glass aquaria containing 6L de-chlorinated tap water. Each aquarium contains 30 experimental animals. Snails and fish were exposed for 24h in the case of *L. acuminata* and 96h for fish *C. punctatus* to sub lethal doses 0.76 mg/L and 1.35 mg/L (40% and 80% of LC_{50} 24h of *L. acuminata)* of taraxerol. Control animals were held in similar conditions without any treatment. After completion of treatment the test animals were removed from aquaria, and washed with freshwater. The nervous, hepatopancreas and ovotestis tissue of *L. acuminata* and muscle, liver and gonadal tissue of freshwater fish *C. punctatus* were quickly dissected out in an ice tray and used for biochemical analysis.

In order to see the effect of withdrawal from treatment, both the experimental animals were exposed for 24h in the case of *L. acuminata* and 96h for fish *C. punctatus* to sub-lethal doses 1.35 mg/L (80% of 24h LC_{50} of *L. acuminata*) of taraxerol, following which the test animals were transferred to freshwater. This water was changed every 24h for the next seven days. After this the test animals were removed from aquaria, washed with freshwater and nervous, hepatopancreas and ovotestis tissue of *L. acuminata* and muscle, liver and gonadal tissues of freshwater fish *C. punctatus* were quickly dissected out in an ice tray and all the above mentioned biochemical parameters were estimated.

Each experiment was replicated at least six times and the values have been expressed as mean ±SE of six replicates. Student's 't' test and analysis of variance were applied to locate significant changes (SOKAL AND ROHLF 1973).

BIOCHEMICAL ESTIMATIONS

Protein: Protein levels were estimated according to the method of LOWRY, ROSENBROUGH, FARR, RANDALL (1951) using bovine serum albumin as standard. Homogenates (5 mg/mL, w/v) were prepared in 10%TCA.

Total free amino acids: Estimation of total free amino acid was made according to the method of SPICES (1957). Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at

6000 xg and used for amino acid estimation.

Nucleic acids: Estimation of nucleic acids (DNA and RNA) was performed, by methods of SCHNEIDER (1957) using diphenylamine and orcinol reagents, respectively. Homogenates (1mg/mL, w/v) were prepared in 5% TCA at 900C, centrifuged at 5000 xg for 20 min and supernatant was prepared and used for estimation. Both DNA and RNA have been expressed as μg/mg tissue.

Glycogen: Glycogen was estimated by the Anthrone method of VAN DER VIES (1954) as modified by MAHENDRU AND AGARWAL (1982) for snail *L. acuminata*. In the present experiment 50 mg of tissue were homogenised with 5 mL of cold 5%TCA. The homogenate was filtered and 1.0 mL of filtrate was used for assay.

Pyruvate: Pyruvate level was measured according to FRIEDEMANN AND HAUGEN (1943). Homogenate (50 mg/mL, w/v) was prepared in 10% TCA. Sodium pyruvate was taken as standard.

Lactate: Lactate was estimated according to BARKER AND SUMMERSON (1941), modified by HUCKABEE (1961). Homogenate (50 mg/mL, w/v) was prepared in 10% cold TCA. Sodium lactate was taken as standard.

Protease: Protease activity was estimated by the method of MOORE AND STEIN (1954). Homogenate (50 mg/mL, w/v) was prepared in cold distilled water. Optical density was measured at 570 nm. The enzyme activity was expressed in μmol of tyrosine equivalent/mg protein/h.

Acid and alkaline phosphatase: Activities of acid and alkaline phosphatase were measured by the method of BERGMEYER (1967) and modified by SINGH AND AGARWAL (1983). Tissue homogenate (2% w/v) was prepared in ice cold 0.9% saline and centrifuged at 5000 xg at 0 °C for 15 min. Optical density was measured at 420 nm against a blank, prepared simultaneously. The enzyme activity has been expressed as amount of p-nitrophenol formed/30 min/mg protein in supernatant.

Lactic dehydrogenase: Lactic dehydrogenase activity was measured according to the method of ANONYMOUS (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 has been expressed as nanomol of pyruvate reduced/min/mg protein.

Succinic dehydrogenase: Succinic dehydrogenase activity was measured by the method of ARRIGONI AND SINGER (1962). Homogenate (50 mg/mL, w/v) was prepared in 1 mL of 0.5 M potassium phosphate buffer, pH 7.6 for 5 min in an ice bath. Optical density was measured at 600 nm. Enzyme activity has been expressed as μmol dye reduced/min/mg protein.

Cytochrome oxidase: Cytochrome oxidase activity was measured according to 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 has been expressed in arbitrary units/min/mg of proteins.

Acetylcholinesterase: Acetylcholinesterase was estimated by the method of ELLMAN, COURTNEY, ANDRES AND FEATHERSTONE (1961) as in 0.1 M phosphate buffer in ice bath. Optical density was measured at 412 nm at 25 °C. Enzyme activity expressed in μmol 'SH' hydrolysed/min/mg protein.

RESULTS

Molluscicidal activity: The toxicity of taraxerol was also time and dose-dependent for the freshwater snail *L. acuminata.* There was a significant negative correlation between LC_{50} values and all the exposure periods for 24h or 96h (Table I). Thus with an increase in exposure periods of taraxerol the LC_{50} values show a significant decrease from 1.69 mg/L (24h);> 1.30 mg/L (48h);> 0.86 mg/L (72h);> to 0.74 mg/L (96h) respectively, in case of freshwater snail *L. acuminata.* (Table I).

Table I. Toxicity (LC_{10}, LC_{50} and LC_{90}) of taraxerol against freshwater snail *Lymnaea acuminata* at different time intervals.
Tabla I. Toxicidad (LC_{10} , LC_{50} y LC_{90}) del taraxerol sobre el caracol Lymnaea acuminata *a diferentes intervalos de tiempo.*

Exposure periods	Effective dose	Limits (mg/L) LCL	UCL	Slope value	'g' factor	'f' ratio	Hetero-geneity
24h	LC10=0.98	0.829	1.089				
	LC50=1.69	1.542	1.962	5.397±0.853	0.10	6.33	0.70
	LC90=2.92	2.397	4.232				
48h	LC10=0.68	0.494	0.818				
	LC50=1.30	1.163	1.496	4.629±0.649	0.13	7.14	0.50
	LC90=2.46	1.984	3.776				
72h	LC10=0.49	0.381	0.586				
	LC50=0.86	0.776	0.938	5.347±0.693	0.07	7.66	0.34
	LC90=1.49	1.348	1.759				
96h	LC10=0.41	0.294	0.514				
	LC50=0.74	0.640	0.822	5.152±0.750	0.08	6.87	0.53
	LC90=1.31	1.187	1.542				

Batches of ten snails were exposed to four different concentrations of taraxerol.
Concentrations given are the final concentrations (w/v) in aquarium water.
Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values.
LCL = Lower confidence limit; UCL = Upper confidence limit

The active (moiety), of taraxerol which were effective against freshwater snail *L. acuminata*, would also cause death amongst the fish. Consequently, mixed populations of 10 snails (*L. acuminata*) and 10 fishes (*C. punctatus*) were treated for 24h of the LC_{90} of taraxerol. Up to the LC_{90} doses for snail *L. acuminata* there was no mortality amongst the freshwater fish *C. punctatus*. The doses, which can be used for killing the snails, are safe for fish. This is supported by our observations in a mixed population.

Effect on freshwater target snail: Data of sub-lethal doses of 40% and 80% of LC_{50} (0.76 mg/L & 1.35 mg/L) taraxerol exposure to freshwater snail *L. acuminata* are given in Tables II and III. Exposure of snails to sub-lethal doses of taraxerol for 24h caused significant alterations in nitrogenous and carbohydrate metabolism in different body tissues of the freshwater snail *L. acuminata.* Total protein and nucleic acids (DNA and RNA) levels were significantly reduced, while free amino acid level was significantly enhanced after the exposure to sub-lethal doses in all the body tissues. Acid and alkaline phosphatase activities were significantly reduced, while protease activity was increased after the exposure.

Total protein levels were reduced to 31%, 37% and 28% of controls after exposure to sub-lethal doses of 1.35 mg/L of taraxerol respectively in the nervous, hepatopancreas and ovotestis tissue of *L. acuminata,* respectively. The DNA level was reduced to 41%, 32% and 28% of controls after treatment with 1.35 mg/L of taraxerol in nervous, hepatopancreas and ovotestis tissue of *L. acuminata*, respectively. The RNA level was reduced to 36%, 39% and 28% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis of *L. acuminata*. Total free amino acid levels were induced to 171%, 151% and 174% of controls after treatment with sub-lethal doses of 1.35 mg /L of taraxerol respectively in nervous,

Table II. Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg) level and activity of protease (µmol of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µmol substrate hydrolysed/30 min/mg protein) in nervous (NT), hepatopancreas (HP) and ovotestis (OT) tissues of *Lymnaea acuminata* after exposure to sub-lethal doses of 40% and 80% (0.76 mg/L and 1.35 mg/L) of taraxerol after 24h.

Tabla II. Cambios en los niveles de proteínas, aminoácidos libres y ácidos nucléicos (ADN y ARN) (µg/mg), actividad de proteasa (µmol de equivalentes de tirosina/mg proteína/h) y de fosfatasas ácida y alcalina (µmol de sustrato hidrolizado/30 min/mg proteína) en tejido nervioso (NT), hepatopáncreas (HP) y ovotestis (OT) de Lymnaea acuminata *tras exposición a dosis subletales de 40% y 80% (0,76 mg/L y 1,35 mg/L) de taraxerol tras 24h.*

Parameter	Tissues	Control	40% of LC_{50} (24h) (0.76 mg/L)	80% of LC_{50} (24H) (1.35 mg/L)	7 days of the withdrawal
Protein	NT	64.0±0.18(100)	37.12±0.24(58)	15.48±0.01(31)	156.8±0.71* (98)
	HP	66.0±2.61(100)	37.62±0.91(57)	24.42±0.81 (37)	136.7±1.00* (97)
	OT	71.0±0.53(100)	38.34±0.87(54)	19.88±0.84(28)	131.1±0.45* (96)
Amino acid	NT	31.3±1.14(100)	51.64±1.15(165)	53.52±0.28(171)	29.3±0.23* (103)
	HP	26.8±1.51(100)	38.86±0.38(145)	40.40±1.07(151)	23.7±0.38* (105)
	OT	34.3±0.68(100)	57.62±0.15(168)	59.68±0.14(174)	21.4±0.10* (104)
DNA	NT	76.6±0.29(100)	46.72±0.31(61)	31.40±0.21(41)	136.7±0.69* (96)
	HP	73.8±0.21(100)	49.44±0.33(67)	23.61±0.29(32)	134.4±0.51* (96)
	OT	81.5±0.89(100)	44.01±0.21(54)	22.82±0.51(28)	137.7±0.34* (95)
RNA	NT	52.31±0.38(100)	26.67±0.27(51)	18.83±0.31(36)	99.9±0.28* (97)
	HP	50.21±0.81(100)	30.12±0.77(60)	19.58±0.22(39)	100.0±0.21* (96)
	OT	55.33±0.41(100)	23.79±2.87(43)	15.49±0.58(28)	98.5±0.17* (93)
Protease	NT	0.471±0.051(100)	0.664±0.050(141)	0.706±0.051(150)	0.639±0.061* (108)
	HP	0.492±0.001(100)	0.6834±0.002(139)	0.713±0.001(145)	0.571±0.012* (94)
	OT	0.474±0.007(100)	0.673±0.003(142)	0.725±0.004(153)	0.739±0.154* (106)
Acid phosphatase	NT	0.281±0.008(100)	0.258±0.006(92)	0.210±0.004(75)	0.260±0.0123* (92)
	HP	0.275±0.007(100)	0.264±0.008(96)	0.225±0.009(82)	0.276±0.013* (93)
	OT	0.280±0.008(100)	0.263±0.003(94)	0.215±0.005(77)	0.267±0.012* (93)
Alkaline phosphatase	NT	0.481±0.021(100)	0.384±0.001(80)	0.307±0.003(64)	0.394±0.002* (91)
	HP	0.482±0.004(100)	0.404±0.002(80)	0.307±0.001(68)	0.426±0.003* (92)
	OT	0.475±0.008(100)	0.384±0.001(81)	0.308±0.002(65)	0.403±0.004* (92)

* Significant ($P<0.05$). Student's 't' test was applied between 80% of LC_{50} (24h) and withdrawal groups. Values are mean ±SE of six replicates. Values in parenthesis are percentage changes with control taken as 100%

hepatopancreas and ovotestis of *L. acuminata* (Table II).

Activity of acid phosphatase was inhibited to 75%, 82% and 77% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis. The activity of alkaline phosphatase was reduced to 64%, 68% and 65% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis. The protease activity was increased to 150%, 145% and 153% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in the nervous, hepatopancreas and ovotestis of snail *L. acuminata* (Table II).

Glycogen and pyruvate levels were significantly reduced, while lactate level was significantly enhanced after the exposure to sub-lethal doses in all the body tissues. Lactic dehydrogenase (LDH), cytochrome oxidase and acetyl-

Table III. Changes in glycogen (mg/g), pyruvate (µmol/g), lactate (mg/g) level and activity of LDH (µmol/mg protein/h), SDH (µmol of dye reduced/min/mg protein), cytochrome oxidase (arbitrary unit/min/mg protein) and AChE (µmol 'SH' hydrolysed/min/mg protein) after 24h exposure to sub-lethal doses of 40% and 80% (0.76 mg/L and 1.35 mg/L) of taraxerol in nervous (NT), hepatopancreas (HP) and ovotestis (OT) tissues of snail *Lymnaea acuminata* after 24h.
Tabla III. Cambios en los niveles de glucógeno (mg/g), piruvato (µmol/g), lactato (mg/g) y actividad de LDH (µmol/mg proteína/h), SDH (µmol de tinción reducida/min/mg proteína), citocromo oxidasa (unidad arbitraria/min/mg proteína) y AChE (µmol 'SH' hidrolizado/min/mg proteína) tras 24h de exposición a dosis subletales al 40% y 80% (0,76 mg/L y 1,35 mg/L) de taraxerol en los tejidos nervioso (NT), hepatopáncreas (HP) y ovotestis (OT) del caracol Lymnaea acuminata *tras 24h.*

Parameter	Tissues	Control	40% of LC_{50} (24h) (0.76 mg/L)	80% of LC_{50} (24H) (1.35 mg/L)	7 days of the withdrawal
Glycogen	NT	6.8±0.02 (100)	3.12±0.03 (46)	2.31±0.01 (34)	7.3±0.03* (93)
	HP	7.2±0.10 (100)	3.67±0.01 (51)	2.80±0.03 (39)	6.8±0.02* (94)
	OT	9.1±0.08 (100)	4.09±0.03 (45)	3.00±0.01 (33)	7.3±0.04* (93)
Pyruvate	NT	0.578±0.03 (100)	0.196± 0.04 (34)	0.167± 0.23 (29)	0.635±0.27* (91)
	HP	0.609±0.03 (100)	0.255±0.27 (42)	0.237±0.11 (39)	0.592±0.08* (90)
	OT	0.581±0.04 (100)	0.180± 0.18 (31)	0.156± 0.27 (27)	0.628±0.03* (92)
Lactate	NT	3.11± 0.07 (100)	5.06± 0.15 (163)	5.56± 0.19 (179)	2.46±0.04* (113)
	HP	3.79±0.05 (100)	6.59± 0.06 (174)	7.20± 0.02 (190)	2.83±0.05* (118)
	OT	3.95± 0.07 (100)	6.47± 0.08 (164)	7.11± 0.06 (180)	2.46±0.03* (113)
LDH	NT	0.084±0.004 (100)	0.068±0.001 (81)	0.043±0.002 (52)	0.065±0.003* (90)
	HP	0.096±0.003 (100)	0.081±0.002 (85)	0.056±0.001 (59)	0.068±0.004 ((91)
	OT	0.087±0.004 (100)	0.072±0.001 (83)	0.043±0.004 (50)	0.070±0.003* (90)
SDH	NT	31.01±0.11 (100)	39.69± 0.25 (128)	51.16±0.31* (165)	18.38±0.84* (112)
	HP	43.21±0.13 (100)	53.58±0.29* (124)	65.24±0.27* (151)	15.63±0.18* (108)
	OT	27.32±0.10 (100)	34.42±0.30* (126)	44.25±0.34* (162)	20.43±0.20* (111)
Cytochrome oxidase	NT	18.23± 0.12 (100)	11.84± 0.16 (65)	10.02±0.18 (55)	16.68±0.04* (92)
	HP	16.21± 0.14 (100)	11.34± 0.21 (70)	10.05±0.31 (62)	13.05±0.12* (90)
	OT	17.32± 0.16 (100)	11.77±0.15 (68)	10.04± 0.27 (58)	16.18±0.02* (94)
AChE	NT	0.068±0.0008 (100)	0.044±0.0003 (65)	0.028±0.0003 (92)	0.064±0.007* (90)
	HP	0.088±0.0002 (100)	0.060±0.0007 (69)	0.036±0.0002 (41)	0.085±0.002* (93)
	OT	0.071±0.0002 (100)	0.046±0.0003 (66)	0.030±0.0004 (43)	0.063±0.007* (90)

Details are as given in Table II

cholinesterase (AChE) activities were significantly reduced, while succinic dehydrogenase (SDH) activity was increased after the exposure.

Glycogen level was reduced to 34%, 39% and 33% of controls after treatment with sub-lethal doses of 1.35 mg/L taraxerol respectively in nervous, hepatopancreas and ovotestis tissues of *L. acuminata*. Pyruvate level was reduced to 29%, 39% and 27% of controls after treatment with sub-lethal doses of 1.35 mg/L taraxerol respectively in nervous, hepatopancreas and ovotestis tissues of *L. acuminata*. Lactate level was increased to 179%, 190% and 180% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis tissues of snail *L. acuminata* (Table III).

Lactic dehydrogenase activity was reduced up to 52%, 59% and 50% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and

Table IV. Changes in total protein, total free amino acids, nucleic acid (DNA & RNA) (µg/mg) level and activity of protease (µmol of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µmol substrate hydrolysed/30 min/mg protein) in muscle, liver and gonadal tissues of freshwater fish *Channa punctatus* after 96h exposure to sub-lethal doses of 40% and 80% (0.76 mg/L and 1.35 mg/L) of taraxerol and 7 days after withdrawal.

Tabla IV. Cambios en los niveles de proteínas, aminoácidos libres, ácidos nucléicos (ADN y ARN) (µg/mg), actividad de la proteasa (µmol of equivalentes de tirosina/mg proteína/h) y fosfatasas ácida y alcalina (µmol sustrato hidrolizado/30 min/mg proteína) en los tejidos muscular, hepático y gonadal del pez Channa punctatus *tras 96h de exposición a dosis subletales al 40% y 80% (0,76 mg/L and 1,35 mg/L) de taraxerol y a los 7 días de retirar el tratamiento.*

Parameter	Tissues	Control	40% of LC_{50} (24h) (0.76 mg/L)	80% of LC_{50} (24H) (1.35 mg/L)	7 days of the withdrawal
Protein	Muscle	171.2±0.75(100)	104.3±0.22(61)	70.1±0.24(41)	162.6±0.68* (95)
	Liver	151.0±0.65(100)	128.3±0.21(85)	119.2±0.20(79)	146.4±1.00* (97)
	Gonadal	144.6±1.00(100)	112.7±0.12 (78)	76.4±0.07(53)	134.4±0.43* (93)
Amino acid	Muscle	38.20±0.23(100)	42.7±0.26(112)	48.5±0.38(127)	42.2±0.20* (110)
	Liver	22.6±0.40(100)	26.8±0.44(119)	31.8±0.40(141)	24.4±0.30* (108)
	Gonadal	31.0±0.70(100)	42.4±0.02(137)	46.1±0.03(149)	35.6±0.08* (115)
DNA	Muscle	152.44±0.73(100)	124.8±0.14(82)	94.4±0.14(62)	144.7±0.60* (96)
	Liver	149.02±0.70(100)	129.8±0.07(87)	105.9±0.13(71)	137.4±0.50* (92)
	Gonadal	143.00±0.73(100)	111.05±0.44(78)	82.94±0.23(58)	128.7±0.30* (90)
RNA	Muscle	103.00±0.28(100)	84.41±0.03(82)	69.01±0.04(67)	99.90±0.28* (97)
	Liver	100.0±0.29(100)	92.00±0.18(92)	72.00±0.18(72)	95.00±0.21* (95)
	Gonadal	106.60±0.61(100)	83.21±0.40(78)	65.02±0.28(61)	98.07±0.16* (92)
Protease	Muscle	0.598±0.011(100)	0.825±0.047(138)	0.926±0.015(155)	0.568±0.060* (92)
	Liver	0.652±0.016(100)	0.796±0.017(122)	0.920±0.014(141)	0.046±0.010* (99)
	Gonadal	0.601±0.016(100)	0.775±0.013(129)	0.949±0.014(158)	0.552±0.154* (92)
Acid phosphatase	Muscle	0.302±0.012(100)	0.138±0.010(30)	0.090±0.013(30)	0.277±0.0122*(92)
	Liver	0.292±0.014(100)	0.110±0.010(38)	0.078±0.009(27)	0.277±0.009* (95)
	Gonadal	0.281±0.015(100)	0.092±0.007(33)	0.061±0.017(22)	0.275±0.010* (98)
Alkaline phosphatase	Muscle	0.451±0.010(100)	0.193±0.006(43)	0.121±0.03(27)	0.410±0.002* (91)
	Liver	0.400±0.030(100)	0.148±0.006(37)	0.096±0.006(24)	0.368±0.002* (92)
	Gonadal	0.438±0.012(100)	0.210±0.005 (48)	0.135±0.003(31)	0.420±0.003* (96)

*, Significant ($P<0.05$) when Student's 't' test was applied between 80% of LC50 (24h) and withdrawal groups
Details are as given in Table II

ovotestis tissue of snail *L. acuminata*. Activity of cytochrome oxidase was reduced to 55%, 62% and 58% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis of *L. acuminata*. Acetylcholinesterase activity was reduced to 92%, 41% and 43% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis of snail *L. acuminata*. The succinic dehydrogenase (SDH) activity was increased to 165%, 151% and 162% of controls after treatment with sub-lethal doses of 1.35 mg/L of taraxerol respectively in nervous, hepatopancreas and ovotestis tissues of freshwater snail *L. acuminata* (Table III).

Effect on freshwater non-target fish: Higher doses (LC_{90} of snails) have no apparent toxic effect on non-target freshwater fish *C. punctatus* after 24h exposure. But exposure of fish to sub-lethal doses

Table V. Changes in glycogen (mg/g), pyruvate (µmol/g), lactate (mg/g) level and activity of LDH (µmol/mg protein/h), SDH (µmol of dye reduced/min/mg protein), cytochrome oxidase (arbitrary unit/min/mg protein) and AChE (µmol 'SH' hydrolysed/min/mg protein) in muscle, liver and gonadal tissues of *Channa punctatus* after 96h exposure to sub-lethal doses of 40% and 80% (0.76 mg/L and 1.35 mg/L) of taraxerol and 7 days after withdrawal.

Tabla V. Cambios en los niveles de glucógeno (mg/g), piruvato (µmol/g), lactato (mg/g) y actividad de LDH (µmol/mg proteína/h), SDH (µmol de tinción reducida/min/mg proteína), citocromo oxidasa (unidad arbitraria/min/mg proteína) y AChE (µmol 'SH' hidrolizado/min/mg proteína) en los tejidos muscular, hepático y gonadal del pez Channa punctatus *tras 96h de exposición a dosis subletales al 40% y 80% (0,76 mg/L and 1,35 mg/L) de taraxerol y a los 7 días de retirar el tratamiento.*

Parameter	Tissues	Control	40% of LC_{50} (24h) (0.76 mg/L)	80% of LC_{50} (24H) (1.35 mg/L)	7 days of the withdrawal
Glycogen	Muscle	2.20±0.001(100)	1.89±0.004(86)	1.38±0.04(63)	1.91±0.02* (87)
	Liver	2.98±0.002(100)	2.17±0.001(73)	2.62±0.03(68)	2.47±0.04* (83)
	Gonadal	3.00±0.01(100)	2.61±0.02(87)	2.13±0.04(71)	2.73±0.04* (91)
Pyruvate	Muscle	3.416±0.018(100)	1.702±0.013(50)	1.097±0.024(32)	3.050±0.014* (90)
	Liver	4.076±0.018(100)	2.446±0.035(60)	1.548±0.007(38)	3.788±0.031* (93)
	Gonadal	2.993±0.036(100)	1.705±0.014(57)	0.921±0.020(31)	2.721±0.016* (91)
Lactate	Muscle	3.816±0.018(100)	4.649±0.090(122)	6.846±0.060(179)	4.221±0.080* (111)
	Liver	2.323±0.020(100)	3.205±0.020(138)	3.750±0.074 (172)	2.500±0.069* (112)
	Gonadal	3.816±0.083(100)	4.502±0.088(118)	6.181±0.092(162)	4.159±0.043* (109)
LDH	Muscle	425.3±0.88(100)	374.2±0.81(88)	259.4±0.83(61)	382.5±0.81* (90)
	Liver	555.0±1.0(100)	521.7±0.80(94)	394.0±0.81(71)	516.1±0.76* (93)
	Gonadal	467.1±0.84(100)	420.3±0.70(90)	350.3±0.81(75)	448.4±0.78* (93)
SDH	Muscle	59.4±0.21(100)	72.4±0.20(122)	81.3±0.27(137)	64.7±0.20* (109)
	Liver	62.2±0.20(100)	72.2±0.20(116)	85.9±0.19(138)	69.3±0.20* (111)
	Gonadal	64.4±0.26(100)	85.8±0.16(110)	95.9±0.10(149)	68.2±0.23* (106)
Cytochrome oxidase	Muscle	28.91±0.21(100)	24.86±0.20(86)	17.92±0.24(62)	27.17±0.30* (94)
	Liver	23.12±0.05(100)	17.10±0.20(74)	15.95±0.24(69)	21.03±0.022* (91)
	Gonadal	33.20±0.05(100)	32.20±0.10(87)	24.28±0.13(73)	30.54±0.17* (92)
AChE	Muscle	0.091±0.0010(100)	0.045±0.0004(50)	0.034±0.0006 (38)	0.085±0.0002* (94)
	Liver	0.097±0.0009(100)	0.053±0.0004(55)	0.034±0.0003(36)	0.082±0.0001* (92)
	Gonadal	0.088±0.0020(100)	0.043±0.0006(49)	0.024±0.0005(27)	0.079±0.0004* (90)

*, Significant ($P<0.05$) when Student's 't' test was applied between 80% of LC50 (24h) and withdrawal groups
Details are as given in Table II

(i.e. 40% and 80% of 24h LC_{50} of snail) 0.76 mg/L and 1.35 mg/L of taraxerol for 96h caused a significant alteration in nitrogenous and carbohydrates metabolism in different body tissues of fish *C. punctatus* (Tables IV and V).

Total protein and nucleic acids (DNA and RNA) levels were significantly reduced, while free amino acid level was significantly enhanced after the exposure to sub-lethal doses in all the studied body tissues. Acid and alkaline phosphatase activities were significantly reduced, while protease activity was increased after the exposure. Total protein levels were reduced to 41%, 79% and 53%; DNA level was reduced to 62%, 71% and 58% and RNA level was reduced to 67%, 72% and 61% in muscle, liver and gonadal tissue of freshwater fish *C. punctatus*. Total free amino acid levels were induced to 127%, 141% and 149% of controls after 96h treatment with 1.35 mg/L of LC_{50} of taraxerol in

muscle, liver and gonadal tissues, respectively (Table IV).

Activity of acid phosphatase was inhibited to 30%, 27% and 22% and activity of alkaline phosphatase was reduced to 27%, 24% and 31% but Protease activity was increased to 155%, 141% and 158% of controls after 96h treatment with 1.35 mg/L of taraxerol in muscle, liver and gonadal tissues of freshwater non-target fish *C. punctatus*, respectively (Table IV).

Glycogen and pyruvate levels were significantly reduced, while lactate level was significantly enhanced after the exposure to sub-lethal doses in the studied body tissues. Lactic dehydrogenase (LDH), cytochrome oxidase and acetylcholinesterase (AChE) activities were significantly reduced, while succinic dehydrogenase (SDH) activity was increased after the exposure. Glycogen level was reduced to 63%, 68% and 71% and pyruvate level was reduced to 32%, 38% and 31% in muscle, liver and gonadal tissue of fish. Lactate level was increased to 179%, 172% and 162% of controls after 96h treatment with 1.35 mg/L taraxerol in muscle, liver and gonadal tissues of fish *c. punctatus*, respectively (Table V).

Lactic dehydrogenase (LDH) activity was reduced to 61%, 71% and 75% and activity of cytochrome oxidase was reduced to 62%, 69% and 73% and acetylcholinesterase (AChE) activity was reduced to 38%, 36% and 27% in muscle, liver and gonadal tissue of fish *C. punctatus*, respectively. Succinic dehydrogenase (SDH) activity was increased to 137%, 138% and 149% of controls after 96h treatment with 1.35 mg/L LC_{50} taraxerol in muscle, liver and gonadal tissues of fish *C. punctatus*, respectively (Table V).

DISCUSSION

It is clear from the results section that the extracted compound taraxerol from the stem-bark of *Codiaeum variegatum* has potent molluscicidal activity against the freshwater target snail *L. acuminata*.

More important is the fact that the extracted compound is much more toxic than synthetic pesticides. The present study demonstrated that taraxerol has higher molluscicidal activity than any of the prevalent synthetic molluscicides like carbamate, organophosphate and synthetic pyrethroids. Thus, the 24h LC_{50} of mexacarbamate (3.5 ppm), aldicarb (30.00 ppm), farmothion (27.00 ppm), cypermethrin (2.5 ppm), permethrin (0.82 ppm) and fenvalerate (2.5 ppm) against *L. acuminata* (SINGH AND AGARWAL, 1981; SINGH AND AGARWAL, 1986; SINGH AND AGARWAL, 1988; SINGH AND AGARWAL, 1991) is higher than that of taraxerol (0.183 ppm), which is about 65 times stronger than the standard molluscicide niclosamide (LC_{50} 11.8 ppm) SAHAY, SINGH AND AGARWAL (1991).

Statistical analysis of the data on toxicity brings out several important points. The χ^2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables (e.g. resistance) do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The slope is thus, an index of the susceptibility of the target animal to the extract used. A steep slope is also indicative of rapid absorption and onset of effects. Since the LC_{50} of taraxerol is within the 95% confidence limits, it is obvious that in replicate tests of random samples, the concentration response lines would fall in the same range (RAND AND PETROCELLI, 1988).

The depletion of protein fraction in different tissues of snail and fish may have been due to their degradation and possible utilization of degraded products for metabolic purposes. MOMMENSEN AND WALSH (1992) reported that proteins are mainly involved in the architecture of the cell, which is the main source of nitrogenous metabolism, and during chronic periods of stress they are also a source of energy. Increment in free amino acids level was the result of breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis. Inhibi-

tions of DNA synthesis might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery (NORDENKJOLD, SODERHALL AND MOLDEUS, 1979).

The increase in the protease activity corroborates the enhancement in the FAA (Free amino acids) level in tissues, the formation of which might be the result of protein hydrolysis in the tissues suggesting stimulation during toxic stress. Similar trend of results on protease activity were also reported by several workers in different animals (*T. mossambica* (Peters), *P. globosa* (Swaimson) including mammals (KABEER, SAHIB, SIVA PRASAD AND SAMBASIVA RAO, 1984). SINGH AND AGARWAL (1992) reported that several euphorbious plants significantly reduced the alkaline and acid phosphatase activity in nervous tissue of *L. acuminata* so the reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis (PILO, ASNANI AND SHAH, 1972) and other secretory activities (IBRAHIM, HIGAZI AND DEMIAN, 1974).

Carbohydrates reserves were depleted to meet energy demand, thus depletion of glycogen may be due to direct utilization for energy generation, a demand caused by active moiety-induced hypoxia. The glycogenolysis seems to be the result of increased secretion of catecholamine due to stress. Decrease in pyruvate level is due to higher energy demand during exposure, which suggests the possibility of a shift towards anaerobic dependence due to a remarkable drop in the amount of oxygen. The increase in lactate also suggests a shift towards anaerobiosis as a consequence of hypoxia leading to respiratory distress (SIVA PRASADA RAO, 1980).

Lactic dehydrogenase (LDH) catalyzes the inter-conversions of lactic acid and pyruvic acid during anaerobic conditions. Inhibition of lactic dehydrogenase and cytochrome oxidase activity indicates that taraxerol significantly inhibits aerobic, as well as anaerobic metabolism in exposed animals (EVERSE AND KALPAN, 1973). Succinic dehydrogenase (SDH) is one of the active regulatory enzymes of the TCA cycle. Inhibition in cytochrome oxidase activity by taraxerol moieties supports that Euphorbiales show a profound impact on the oxidative metabolism.

Withdrawal experiments were performed to see whether biochemical alteration caused by taraxerol moiety would return to normal, if the treatment were discontinued. There was nearly complete recovery of total protein, total free amino acid, lactate, nucleic acid (DNA and RNA), pyruvate level and in the activity of cytochrome oxidase, succinic dehydrogenase, protease, lactic dehydrogenase, acetylcholinesterase and acid and alkaline phosphatase but only a partial recovery of glycogen level in the different body tissues of freshwater snail *L. acuminata* and fish *C. punctatus.*

In conclusion, it is believed that extracted compound of selected plants may be used as a potent source of molluscicides for addition; plant products are less expensive, easily available, easily soluble in water and safer for non-target animals than synthetic molluscicides.

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