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Original Article

EFFICACY OF BINARY COMBINATION OF DELTAMETHRIN+MGK-264 ON LEVELS OF BIOCHEMICAL CHANGES IN THE SNAIL *LYMNAEA ACUMINATA*

R. N. SINGH[a*], PRADEEP KUMAR[b], NAVNEET KUMAR[a], D. K. SINGH[c]

[a*]Department of Zoology, Maharana Pratap P. G. College Jungle Dhusan, Gorakhpur (U. P), [b]Department of Zoology, S. G. N. Govt. P. G. College Muhammadabad Gohna Mau, (U. P), [c]Department of Zoology, D. D. U Gorakhpur University Gorakhpur, (U. P)
Email: rnsingh6010@gmail.com

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ABSTRACT

Objective: The objective of the present study is the evaluation of the effect of the sublethal (40% and 60% of 48h LC_{50}) binary combination (1:5 ratios) of molluscicides deltamethrin+MGK-264 on the endogenous levels of protein, amino acid and nucleic acid in different tissues of snail *Lymnaea acuminata*.

Methods: The snails were treated with 1:5 mixtures of sub-lethal concentration of (40% and 60% of 48h LC_{50}) deltamethrin+MGK-264 on the protein, amino acid and nucleic acid levels in gonadal, nervous and foot tissue of *L. acuminata*. In order to study the effect of withdrawal from treatment, the snails were first exposed to the above concentrations for 96h, after which they were transferred to freshwater. Water was changed every 24h for the next seven days, after which different biochemical parameters were estimated.

Results: There was a significant change in the levels of protein (sublethal concentration of 60% of 48h LC_{50} after 96h) gonadal, nervous and foot tissues are 48.1, 12.1 and 14.5%, respectively, amino acid are 273, 234 and 252%, respectively, DNA are 25.1, 38.9 and 42.1%, respectively and RNA are 12.2, 30.7 and 30.5%, respectively. These changes were time and concentration-dependent. In the withdrawal experiment, the snails were treated for 96h to transfer in freshwater for 7 d, which caused significant recovery in all the biochemical parameters.

Conclusion: The present study concluded that the high molluscicidal activity of deltamethrin+MGK-264 simultaneous decrease in the levels of proteins, DNA, RNA and increase in the level of amino acids.

Keywords: Fasciolosis, Deltamethrin+MGK-264, Protein, Amino acid, Nucleic acid, *Lymnaea acuminata*

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INTRODUCTION

Digenetic trematode *Fasciola gigantica* is a parasite of great importance in eastern Uttar Pradesh, which causes endemic fasciolosis in cattle and livestock in northern part of India [1]. Snail *Lymnaea acuminata* is the intermediate host of liver fluke *F. gigantica*. An effective method to control the fasciolosis is to reduce the population of vector snails and break the life cycle of fluke [2-8]. Indiscriminate use of synthetic pyrethroids against the different pests control has created the problem of acute and chronic toxicity to man and other non-target animals [9]. The exposure of pyrethroids has been widely documented in humans, including exposure of pregnant women, infants and children [10-13]. Primary mode of action of pyrethroid is on voltage-sensitive sodium channels in nerve cell [9, 14]. The acute mammalian neurotoxicity of pyrethroid has been well characterized and several comprehensive reviews on pyrethroid toxicity, metabolism and actions are available [9, 15, 16]. As a group synthetic pyrethroids causes high pest mortality at lower concentration [17-19] and are less toxic to the non-target animal, especially mammal [20]. Singh and Agarwal [21], Singh and Agarwal [22] studied the effect of permethrin cypermethrin on the snail *L. acuminata.* Singh *et al.* [4] has studied the effect of sub-lethal, and deltamethrin binary combination of deltamethrin+MGK-264 on the levels of phospholipids and lipid peroxidation in the nervous tissue of snail *L. acuminata.* Present study is the continuation of the same work to observe the effect of the sub-lethal binary combination of deltamethrin+MGK-264 on the endogenous levels of protein, amino acid, nucleic acid in different tissues of the snail *L. acuminata*.

MATERIALS AND METHODS

Adult *L. acuminata* (2.25±0.20 cm in length) were collected locally and used as experimental animals from lakes and low lying submerged fields in Gorakhpur. The snails were acclimatized in laboratory conditions for 72h in dechlorinated tap water at 25±1 °C. Batches of 50 snails were kept in glass aquaria containing 5L dechlorinated tap water. Snails were treated according to the method of Singh and Agarwal [21, 23]. During the course of the experiment no food was given to treated snails. In control group no mortality in snail population was noted. The oxygen concentration was normal in control animals as there was no sign of oxygen deficiency stress. The test method was standardized in our laboratory in which 40 snails/5L water was used, which causes no oxygen stress in test snails [21, 23]. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide, and bicarbonate alkalinity were 6.5-7.3 mg/l, 5.2-6.3 mg/l and 103.0-105.0 mg/l, respectively. Dead snails were removed immediately from aquaria to avoid any contamination.

Drug solutions

Solution of desire strength of deltamethrin [(s)-α-cayno-3-phenoxybenzyl (IR, 3R)-3-(2,2-dibromoviny)-2,-1/*m*-dimethyl-cyclopropane-carboxylate] (Evid and Company Chemicals Ltd. India) and MGK-264 [*N*-(2-ethylhexyl)-8,9,10-trinorborn-5ene-2,3-dicarboximide] (McLaughlin Gormley King Co. USA) were prepared in acetone. Appropriate amounts of the deltamethrin and MGK-264 were mixed in 1:5 ratios, respectively and added to the aquarium water.

Treatment of snails

Snails were maintained and treated with pesticide deltamethrin+MGK-264 according to the method of Singh and Agarwal [23]. Adult *L. acuminata* were kept in glass aquaria containing 5L dechlorinated tap water. Each aquarium contains 50 snails. The snails were treated with 1:5 mixtures of deltamethrin and MGK 40% (0.0013+0.0065 mg/l) and 60% (0.002+0.01 mg/l) of 48h LC_{50} as reported by Sahay and Agarwal [24]. The control snails were treated with a single concentration of MGK-264 0.01 mg/l for 24h, 48h, 72h and 96h. This was the highest amount of MGK-264 present in the various deltamethrin and MGK-264 mixture.

Withdrawal experiment

In order to study the effect of withdrawal from treatment, the snails were first exposed to the above concentrations for 96h, after which they were transferred to freshwater. Water was changed every 24h for the next seven days, after which different biochemical parameters were estimated.

Biochemical estimations

Biochemical estimation was made in the gonadal, nervous and foot tissues of experimental snails. Treated/control snails were removed from the aquaria, their gonadal, nervous and foot tissues were dissected out, the adherent tissue was removed and the organs were put on a filter paper for the absorption of water. 15-20 snails had to be dissected in order to obtain 100 mg nervous tissue while there was enough gonadal and foot tissue in the single snail.

Estimation of protein

Quantitative estimation of protein was made according to the method of Lowry *et al.* [25]. The gonadal, nervous and foot tissues were homogenized (1 mg/ml, w/v) in 10% TCA using an electrical homogenizer for 5 min. Standard curves were prepared with different concentrations of bovine serum albumin. Results have been expressed as µg/mg tissues.

Estimation of amino acid

Method of Spies [26] was used to estimate total free amino acids. The gonadal, nervous and foot tissues were homogenized in 96% ethanol (10:1 w/v) in an electrical homogenizer for 5 min and centrifuged at 8000g for 20 min. In 0.1 ml of supernatant, 0.1 ml of distilled water and 2.0 ml of ninhydrin reagent was added and mixed thoroughly. The reaction mixture was kept in boiling water bath for exactly 15 min. Two milliliters of 50% ethanol was added to the above solution after cooling. A violet colour developed which was measured at 575 nm. Glycine was used to determine the standard curves. Free amino acids have been expressed as µg/mg tissues.

Estimation of nucleic acids

Estimation of nucleic acids (DNA and RNA) was performed by the method of Schneider [27]. Homogenate (1.0 mg/ml, w/v) of gonadal, nervous and foot tissues were prepared in 5% TCA at 90 °C. The result suspension centrifuged (5000g x 20 min) and clear supernatant solution were taken for the nucleic acids estimation. Standard curves were drawn at different concentrations of calf thymus DNA to determine the standard curve. Yeast RNA was used to determine the standard. Results have been expressed as µg/mg tissue.

Statistical analysis

The data have been expressed as mean±SE of at least six replicates. Student's "t" test was applied between controls and treated groups to analyzed significant changes ($P<0.05$), two-way analysis of variance was applied to detect the effect of concentration and time on biochemical responses [28].

Table 1: Changes in the level of total protein exposure to 40% (0.0013 mg/l Deltamethrin+0.0065 mg/l MGK-264) and 60% (0.002 mg/l Deltamethrin+0.01 mg/l MGK-264) 48 LC_{50} of Deltamethrin+MGK for *L. acuminata* in the gonadal, nervous and foot tissue after exposure for 24h, 48h, 72h and 96h. In vase of snail effect of withdrawal for 7 d exposure for 96h has also been given, total protein (µg/mg tissue)**

L. acuminata	Control	24h		48h		72h		96h		Effect of withdrawal after 7 d	
		40%	60%	40%	60%	40%	60%	40%	60%	40%	60%
Gonadal tissue	83.90±0.68 (100)	81.26±0.82* (96.9)	75.26±1.77* (89.5)	70.21±1.42* (83.7)	62.99±1.66* (75.1)	61.26±1.62* (73.0)	62.19±1.35* (162.2)	52.94±1.60* (63.1)	40.35±1.61* (48.1)	80.14±1.78+ (95.52)	78.56±1.20+ (93.64)
Nervous tissue	84.92±1.78 (100)	77.55±1.16* (91.70)	68.29±1.64* (80.40)	67.62±1.17* (79.60)	59.53±1.25* (70.10)	44.79±1.01* (52.70)	40.10±0.91* (47.20)	24.80±1.29* (29.20)	10.23±1.28* (12.10)	83.16±1.75+ (97.92)	80.23±1.39+ (94.47)
Foot tissue	59.48±1.61 (100)	51.39±0.94* (86.39)	46.33±1.47* (77.89)	29.42±1.22* (66.27)	36.14±1.21* (60.76)	24.68±1.41* (41.49)	20.17±1.06* (33.91)	13.21±1.17* (22.21)	8.64±0.99* (14.5)	58.55±1.38+ (98.44)	55.47±1.65+ (93.26)

Values are mean ±SE of six replicates. Values in parentheses indicate percent change with controls taken as 100%, *Significantly ($P<0.05$) different from controls then student's t-test was applied,+Significantly ($P<0.05$) different from 40% and 60% 96h treated groups when student's t-test was applied, Two-way analysis of variance demonstrated that, in snails, changes were dose and time-dependent, **100 mg of various tissues were used for each estimation in case of snails

Table 2: Changes in the level of total free anino acid exposure to 40% (0.0013 mg/l Deltamethrin+0.0065 mg/l MGK-264) and 60% (0.002 mg/l Deltamethrin+0.01 mg/l MGK-264) 48 LC_{50} of deltamethrin+MGK for *L. acuminata* in the gonadal, nervous and foot tissue after exposure for 24h, 48h, 72h and 96h. In vase of snail effect of withdrawal for 7 d exposure for 96h has also been given, the total free amino acid (µg/mg tissue)**

L. acuminata	Control	24h		48h		72h		96h		Effect of withdrawal after 7 d	
		40%	60%	40%	60%	40%	60%	40%	60%	40%	60%
Gonadal tissue	28.87±0.95 (100)	32.83±0.60* (113.7)	37.47±0.81* (129.8)	46.09±0.93* (159.7)	55.55±1.16* (192.4)	51.85±1.13* (179.6)	68.35±1.13* (236.8)	60.90±1.05* (210.9)	78.89±1.07* (273.0)	40.70±0.58+ (102.87)	31.17±0.23+ (107.97)
Nervous tissue	33.39±1.58 (100)	39.07±0.66* (117.0)	43.59±0.57* (130.6)	46.06±0.86* (137.9)	50.02±0.96* (149.8)	57.66±0.92* (172.70)	64.68±0.99* (193.70)	63.28±1.21* (189.50)	78.26±0.85* (234.0)	34.95±1.03+ (104.67)	37.96±1.12+ (113.68)
Foot tissue	29.88±1.04 (100)	34.09±0.94* (114.1)	39.13±0.92* (131.9)	40.71±1.05* (136.2)	49.06±0.97* (164.2)	50.56±0.91* (169.2)	62.26±1.08* (208.4)	63.03±1.10* (210.09)	75.48±1.14* (252.0)	31.93±1.06+ (106.86)	32.71±1.05+ (109.47)

Values are mean ⊥SE of six replicates. Values in parentheses indicate percent change with controls taken as 100%, *Significantly ($P<0.05$) different from controls then student's t-test was applied,+Significantly ($P<0.05$) different from 40% and 60% 96h treated groups when student's t-test was

applied, Two-way analysis of variance demonstrated that, in snails, changes were dose and time-dependent, **100 mg of various tissues were used for each estimation in case of snails.

Table 3: Changes in the level of DNA exposure to 40% (0.0013 mg/l Deltamethrin+0.0065 mg/l MGK-264) and 60% (0.002 mg/l Deltamethrin+0.01 mg/l MGK-264) 48 LC_{50} of deltamethrin+MGK for *L. acuminata* in the gonadal, nervous and foot tissue after exposure for 24h, 48h, 72h and 96h. In vase of snail effect of withdrawal for 7 d exposure for 96h has also been given, DNA level (µg/mg tissue)**

L. acuminata	Control	24h		48h		72h		96h		Effect of withdrawal after 7 d	
		40%	60%	40%	60%	40%	60%	40%	60%	40%	60%
Gonadal tissue	74.26±1.99 (100)	66.66±1.65* (89.77)	60.75±1.72* (81.81)	58.33±1.62* (78.55)	47.24±1.37* (63.61)	43.82±1.57* (59.01)	36.42±1.28* (49.04)	30.39±1.63* (40.91)	19.22±1.47* (25.1)	72.98±1.56+ (98.28)	68.94±1.68+ (92.84)
Nervous tissue	36.36±0.64 (100)	33.60±0.89* (92.4)	31.92±0.85* (87.8)	25.34±1.47* (69.7)	22.71±1.27* (62.5)	20.90±1.26* (57.50)	16.53±1.53* (45.5)	16.13±1.28* (44.40)	14.20±0.93* (38.90)	35.74±1.23+ (98.32)	33.67±0.98+ (92.63)
Foot tissue	26.34±0.79 (100)	23.72±0.09* (90.1)	21.91±1.13* (83.2)	20.63±1.62* (78.3)	19.22±1.01* (72.9)	17.07±1.19* (64.8)	14.04±1.19* (53.3)	13.84±1.28* (52.5)	11.09±1.04* (42.1)	25.87±0.86+ (98.22)	24.99±0.85+ (94.87)

Values are mean ±SE of six replicates. Values in parentheses indicate percent change with controls taken as 100%, *Significantly (P<0.05) different from controls then student's t-test was applied,+Significantly (P<0.05) different from 40% and 60% 96h treated groups when student's t-test was applied, Two-way analysis of variance demonstrated that, in snails, changes were dose and time-dependent, **100 mg of various tissues were used for each estimation in case of snails.

Table 4: Changes in the level of RNA exposure to 40% (0.0013 mg/l Deltamethrin+0.0065 mg/l MGK-264) and 60% (0.002 mg/l deltamethrin+0.01 mg/l MGK-264) 48 LC_{50} of deltamethrin+MGK for *L. acuminata* in the gonadal, nervous and foot tissue after exposure for 24h, 48h, 72h and 96h. In vase of snail effect of withdrawal for 7 d exposure for 96h has also been given, RNA level (µg/mg tissue)**

L. acuminata	Control	24h		48h		72h		96h		Effect of withdrawal after 7 d	
		40%	60%	40%	60%	40%	60%	40%	60%	40%	60%
Gonadal tissue	64.20±1.29 (100)	55.29±1.27* (86.12)	46.14±1.43* (71.87)	44.49±1.48* (69.29)	32.17±1.55* (50.11)	29.35±1.70* (41.04)	19.94±1.75* (31.06)	14.18±1.47* (22.09)	7.80±1.44* (12.20)	62.59±1.60+ (97.49)	59.78±1.61+ (93.01)
Nervous tissue	23.64±0.92 (100)	20.00±0.49* (84.60)	18.12±0.56* (76.60)	16.98±0.51* (71.80)	14.62±0.47* (61.80)	14.02±0.53* (59.30)	11.04±0.57* (46.70)	9.13±0.84* (38.60)	7.26±0.83* (30.70)	22.98±0.59+ (97.71)	22.01±0.73+ (93.10)
Foot tissue	16.80±0.82 (100)	14.19±0.44* (84.50)	12.29±0.51* (73.20)	11.97±0.55* (71.30)	10.30±0.71* (61.30)	9.19±0.66* (54.70)	8.33±0.97* (49.60)	7.14±1.09* (42.50)	5.13±0.75* (30.50)	21.15±0.73+ (96.13)	15.83±0.98+ (94.22)

Values are mean ±SE of six replicates. Values in parentheses indicate percent change with controls taken as 100%, *Significantly (P<0.05) different from controls then student's t-test was applied,+Significantly (P<0.05) different from 40% and 60% 96h treated groups when student's t-test was applied, Two-way analysis of variance demonstrated that, in snails, changes were dose and time-dependent, **100 mg of various tissues were used for each estimation in case of snails.

RESULTS

The level of total protein in untreated snail tissue was 83.9µg/mg in ovotestis, 84.92µg/mg in nervous tissue and 59.48µg/mg in foot tissue (table 1). Exposure of *Lymnaea acuminata* to 40% (0.0013 mg/l deltamethrin+0.0065 mg/l MGK) and 60% (0.002 mg/l deltamethrin+0.01 mg/l MGK) of 48h LC_{50} of a mixture of deltamethrin+MGK-264 caused significant changes in the endogenous levels of protein in ovotestis, nervous and foot tissue of snails (table 1).

Treatment with the 40% of 48h LC_{50} of deltamethrin+MGK for 24h, 48h, 72h and 96h decreased the level of protein in gonadal tissue up to 96.9%, 83.7%, 73.0% and 63.1% of control, respectively (table 1). Likewise, in nervous tissue it decreased to 91.7%, 79.6%, 52.7% and 29.2% of controls and in the foot tissue it decreased to 86.39%, 66.27%, 41.49% and 22.21% of controls after exposure for 24h, 48h, 72h and 96h, respectively (table 1). Exposure to higher concentrations of deltamethrin+MGK (60% of 48h LC_{50}) for the same exposure period caused a further greater decrease in the levels of protein in various tissues of snail (table 1). Two-way analysis of variance demonstrated that a decrease in the levels of proteins was both concentration and time-dependent. Withdrawal of deltamethrin+MGK-264 treated snails for 7 d caused near-complete recovery in the levels of total protein in the gonadal, nervous and foot tissue of *L. acuminata*. In snail treated with 40% of LC_{50} for 96h the total protein levels which have decreased to 63.1%, 29.2% and 22.21% of controls in the gonadal, nervous and foot tissue recovered to 95.52%, 97.92% and 98.44% of the controls in corresponding tissues (table 1). Likewise, after seven days withdrawal period of snails treated with 60% of 48h LC_{50} for 96h, the total protein levels in gonadal, nervous and foot tissue recovered to 93.64%, 94.47% and 93.26% of controls, respectively (table 1).

Exposure for 24h, 48h, 72h and 96h to 40% and 60% of 48h LC_{50} of deltamethrin+MGK-264 caused a significant change in the levels of total free amino acids in the gonadal, nervous and foot tissues of *L. acuminata*. The levels of total free amino acids in untreated snail tissue were 28.87%, 33.39 and 29.88µg/mg, respectively (table 2). *In vivo* treatment with 40% of 48h LC_{50} of drltamethrin+MGK-264 mixture for 24h, 48h, 72h and 96h increased the levels of total free amino acid in gonadal tissue up to 113.7%, 159.7%, 179.6% and 210.9% of controls, respectively. Likewise, in nervous tissue it increased to 117.0%, 137.9%, 172.7% and 189.5% while in foot tissue, this increase was 114.1%, 136.2%, 169.2% and 210.9% of controls, respectively (table 2).

In *in vivo* treatment with 60% of 48h LC_{50} for 24h, 48h, 72h and 96h increased levels in of free amino acids the gonadal tissue to 129.8%,

192.4%, 236.8% and 273.0% of controls, in nervous tissue to 130.6%, 149.8%, 193.7% and 239.0% and in the foot tissue to 131.9%, 164.2%, 208.4% and 252.0% of controls, respectively (table 2). Two-way analysis of variance demonstrated that these increases were time and concentration-dependent. Withdrawal of deltamethrin+MGK-264 treated snails for 7 d caused reduction in the levels of total free amino acid in the gonadal, nervous and foot tissue of *L. acuminata*. In snail treated with 40% of LC_{50} for 96h the total free amino acid levels have increased to 210.9%, 189.5% and 210.9% of controls in the gonadal, nervous and foot tissue returned to 102.87%, 104.67% and 106.86% of the controls in these tissues (table 2). Likewise, after seven days of withdrawal snails treated with 60% of 48h LC_{50} for 96h, the total free amino acid levels in gonadal, nervous and foot tissue came back to 107.97%, 113.68% and 109.47% of controls, respectively (table 2).

Exposure of snails for 24h, 48h, 72h and 96h of 40% and 60% of 48h LC_{50} of deltamethrin+MGK-264 mixture also caused a significant ($P<0.005$) time and concentration-dependent reduction in the levels of total DNA in the ovotestis, nervous and foot tissue of *L. acuminata* (table 3).

Exposure of *L. acuminata* for 24h, 48h, 72h and 96h of 40% of 48h LC_{50} of deltamethrin+MGK-264 mixture reduced the levels of DNA in the ovotestis to 89.77%, 78.55%, 59.01% and 40.91% of controls (table 3). The reduction in nervous tissue was 92.4%, 69.7%, 57.5% and 44.4% and the foot tissue 90.1%, 78.3%, 64.8% and 52.2% of controls (table 3). All these changes were statistically significant ($P<0.005$, table 3), except for changes in the DNA levels in the foot tissue after 24h exposure.

Exposure of to 60% of 48h LC_{50} of deltamethrin+MGK-264 for 24h, 48h, 72h and 96h caused a further decline in the levels of DNA in the ovotestis, nervous and foot tissue of snail *L. acuminata* (table 3). The DNA levels in the ovotestis fell to 81.81%, 63.61%, 49.04% and 25.1% of controls (table 3). In the nervous tissue DNA levels dropped to 87.80%, 62.5%, 45.5% and 38.9% of controls while in the foot tissue the levels became 83.2%, 72.9%, 53.3% and 42.1% of controls after 24h, 48h, 72h and 96h of exposure respectively (table 3). Two-way analysis of variance demonstrated that a decrease in the levels of DNA was both concentration and time-dependent. Snails Withdrawn from treatment for 7 d after 96h treatment with deltamethrin+MGK-264 demonstrated a significant recovery in the levels of total DNA in the gonadal, nervous and foot tissue of *L. acuminata*. In snail treated with 40% of LC_{50} for 96h the total DNA levels which have decreased to 40.91%, 44.40% and 52.50% of controls in the gonadal, nervous and foot tissue, respectively (table 3), returned to 98.28%, 98.32% and 98.22% of the controls in these tissues (table 3). Likewise, in snails treated with 60% of 48h LC_{50} for 96h, the total DNA levels in gonadal, nervous and foot tissue came back to 92.84%, 92.63% and 94.87% of controls, respectively (table 3) after seven days drug withdrawal.

Exposure of snails for 24h, 48h, 72h and 96h to the two concentration of deltamethrin+MGK-264 caused a significant ($P<0.05$) time and concentration-dependent reduction in the total RNA levels in the ovotestis, nervous and foot tissue of *L. acuminata* (table 4).

Exposure of *L. acuminata* for 24h, 48h, 72h and 96h of 40% of 48h LC_{50} of deltamethrin+MGK-264 mixture reduced the levels of RNA to 86.12%, 69.29%, 41.04% and 22.09% of controls in ovotestis, to 84.60%, 71.80%, 59.30% and 38.60% in nervous tissue and 84.6%, 71.3%, 64.7% and 42.5% of controls in the foot tissue (table 4). Exposure to 60% of 48h LC_{50} of deltamethrin+MGK-264 mixture for 24h, 48h, 72h and 96h caused a greater reduction in the three tissues. In the ovotestis RNA levels went down to 71.87%, 50.11%, 31.06% and 12.20% of controls, in nervous tissue to 76.60%, 61.80%, 46.70% and 30.70% and in the foot tissue to 73.2%, 61.3%, 49.6% and 30.5% of controls, respectively (table 4). Two-way analysis of variance demonstrated that these increases were time and concentration-dependent. Seven days after the snails were withdrawn from drug treatment, it was found that there was a significant recovery in the levels of total RNA in the gonadal, nervous and foot tissue of *L. acuminata*. In snail treated with 40% of LC_{50} for 96h the total RNA levels which had decreased to 22.09% 38.60% and 42.50% of controls in the gonadal, nervous and foot tissue, respectively (table 4), returned to 97.49%, 97.21% and 96.13% of controls (table 4). Likewise, in snails treated with 60% of 48h LC_{50} for 96h, the total RNA levels in gonadal, nervous and foot tissue came back to 93.01%, 93.10% and 94.22% of controls, respectively (table 3) after seven days withdrawal from treatment.

DISCUSSION

Single treatment of deltamethrin and binary combination of deltamethrin+MGK-264 are extremely toxic to *Lymnaea acuminata* [29]. Present study is the extension of the same work, in which the efficacy of sublethal concentration of deltamethrin+MGK-264 on different biochemical parameters has been investigated. Thus levels of protein, total free amino acids, DNA and RNA in gonadal, nervous and foot tissue have been studied in treated and untreated *L. acuminata*. It is clear from the result section that exposure of *L. acuminata* to sublethal concentration of deltamethrin+MGK-264 increase the levels of total free amino acid along with the increase in the levels of protein and also decrease the levels of DNA and RNA in gonadal, nervous and foot tissue. Singh and Singh [30] have summarized the presence of lipids in biological membrane. These membranes are rich in polyunsaturated fatty acids. Lipids accounts for 28% to 79% of the mass of cell membranes. While the inner mitochondrial membrane contains about 60% proteins and only 20% lipids, the myelin sheath of membrane contains as much as 80% lipids and only 20% protein [31]. Most snail's membrane, however, contains nearly equal amounts of protein and fat. Membrane lipids are mainly phospholipids most of which are phosphoglycerides. Up to 20% of the phospholipids are acidic, which are negatively charged and are associated with membrane proteins due to lipid-protein interaction [31].

Reduction in the levels of protein and the simultaneous increase in the total free amino acid levels in gonadal, nervous and foot tissue of *L. acuminata* indicate that treatment with deltamethrin+MGK-264 inhibits the synthesis of protein which ultimately results an increase in the free amino acid pool in the cell. Singh *et al.* [32] has reported that deltamethrin increase the free amino acid level with a simultaneous decrease in protein, DNA and RNA levels in the gonadal, nervous and foot tissues of the snail *L. acuminata*. Rao *et al.* [33] have noted a significant reduction in protein levels in fresh water mussel *Lamillidens marginalis* exposed to methyl parathion. Singh *et al.* [34] have reported that the pyrethroids-cypermethrin, permethrin and fenvalerate caused a significant inhibition in the protein levels and enhancement in the amino levels in the foot and hepatopancreas of *Lymnaea acuminata*, which was recovered after withdrawal from the drugs. The synthesis of protein in any of the tissue can be affected in two ways by a chemical, (I) it either affects the RNA synthesis at the transcription stage or (II) it somehow affects the uptake of amino acids in the polypeptide chain. Both these possibilities may account for the lower protein content in the affected tissue. In the first case, the RNA synthesis would be inhibited, resulting in reduced RNA as well as protein content. In the second case, only the protein content would be affected [35]. The result section has indicated that following treatment with deltamethrin+MGK-264 in levels of DNA decrease is time and concentration-dependent. The effect was more pronounced in the gonadal tissue. Thus after treatment with 60% of 48h LC_{50} of deltamethrin+MGK-264 for 96h the DNA levels in gonadal tissue were 25.1% of controls as compared to 38.9% in nervous tissue and 42.1% of foot tissue. DNA is an index of the total cell number, a decrease in DNA content indicates a reduction in the cell number of the tissue being studied [36, 37]. The decrease in DNA content in *Lymnaea acuminata* maybe because of cell death caused by the release of toxic aldehydes resulting from lipid peroxidation [4].

As stated earlier a drop in protein levels could be because of lesser availability of DNA at the translation level. Data emerging from the result section demonstrated that treatment following with sublethal concentration of deltamethrin+MGK-264, there was a sharp decline in the levels of RNA. The changes, however, in case of all the treatment were time and concentration-dependent. With the reduction in DNA levels change in RNA level is certainly not surprising. With reduced

RNA level, there is bound to be a fall in protein levels because of reduction in the synthesis of new protein. In case of neoplastic diseases, DNA change is generally believed to be initial biochemical alteration leading to neoplastic transformation and it is widely accepted that such a genetic alteration is result of covalent interaction of chemical carcinogens with DNA either directly or after metabolic activation by microsomal mixed+function oxidase [38, 39]. In the present study, MGK-264, which is a mixed-function oxidase inhibitor [40, 41] when used with deltamethrin would have prevented metabolic activation of deltamethrin so that it seems that deltamethrin acts directly at the synthesis site of DNA. Chem *et al.* [42] have demonstrated that in rat the treatment of deltamethrin induced a decreased mitochondrial membrane potential and increased permeability, and reduced expression of cytochrome c, thus depressing the activity of cytochrome c oxidase significantly. These finding supports the earlier study of Singh and Agarwal [43], who has demonstrated that type II pyrethroid cypermethrin reduced the activity of cytochrome c oxidase in the nervous tissue of *L. acuminata*.

Data emerging from the result section demonstrated that after seven days of withdrawal from 96h deltamethrin+MGK-264 treatment, there was near-complete recovery in the levels of protein, total free amino acids, DNA and RNA in the gonadal, nervous and foot tissue of *L. acuminata*. This finding demonstrates that the effects of the pyrethroids are reversible. The data emerging from the result section that the biochemical changes brought about by deltamethrin or deltamethrin mixed with the synergist MGK-264 are qualitatively the same and quantitatively very similar. Therefore, it is clear that the job of MGK-264 is to prevent the metabolic oxidation of deltamethrin and it perhaps does not have any independent effect on its own.

CONCLUSION

In conclusion, it can be stated that the high molluscicidal activity of deltamethrin+MGK-264 is due to its effect on the nerve membrane where it alters the configuration of the membrane, through the process of increased in the level of amino acids and a simultaneous decrease in the levels of proteins, DNA and RNA.

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CONFLICT OF INTERESTS

Declared none

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