Molluscicidal Effect of Nicotinanilide and its Intermediate Compounds against a Freshwater Snail *Lymnaea luteola,* the Vector of Animal Schistosomiasis

D Sukumaran+, BD Parashar, AK Gupta, K Jeevaratnam, Shri Prakash

Defence Research & Development Establishment, Jhansi road, Gwalior - 474 002, India

The molluscicidal effect of nicotinanilide was evaluated and compared with niclosamide (2',5-dichloro-4'-nitrosalicylanilide, ethanolamide salt) against different stages of the freshwater snail Lymnaea luteola *i.e., eggs, immature, young mature, and adults. Calculated values of lethal concentrations (LC_{50} and LC_{90}) showed that both nicotinanilide and niclosamide as toxic against eggs, immature, and adults. The young mature stage of the snails was comparatively more tolerant to both molluscicides than the other stages. The toxicity of the intermediate compounds of nicotinanilide against the young mature stage of the snails showed them as ineffective. The mortality pattern of the snails exposed to LC_{90} concentration of these molluscicides showed niclosamide to kill faster (within 8 to 9 h) than nicotinanilide (26 to 28 h).*

In view of the above studies it may be concluded that both molluscicides are toxic against all the stages of the L. luteola *snails.*

Key words: nicotinanilide - niclosamide - molluscicide - *Lymnaea luteola* - animal schistosomiasis - snails as vectors

Schistosomiasis, a dreadful disease caused by parasitic trematode worm in both humans as well as in animals is widespread in the world especially in developing countries (Engels et al. 2002). It is considered second only to malaria as a major target disease of the World Health Organization (Xiao et al. 2002). Various species of freshwater snail act as intermediate hosts of schistosomiasis (Malek & Cheng 1974). The snails belonging to the family Lymnaeidae are known to act as intermediate host of both human and animal fascioliasis (Horak & Kolarova 2001). The freshwater snail *Lymnaea luteola* Lamarck (Mollusca: Gastropoda), is widely distributed in India and acts as intermediate host of *Schistosoma incognitum, S. nasale, Orientobilharzia dattae*, *Fasciola hepatica*, *F. gigantica*, the causative agents of fascioliasis among cattle and cercarial dermatitis in human beings in the Northern part of India (Malek & Cheng 1974, Singh & Agarwal 1981, Jairajpuri 1991, Agrawal et al. 2000).

Control of snails is regarded as one of the best preventive measures in controlling schistosomiasis (Madsen & Christiansen 1992, WHO 1993, Lardans & Dissous 1998) where use of mollusciciding has given very satisfactory results (WHO 1993, Sturrock 1995). Studies on chemical structure – biological activity relationship of various compounds have permitted the identification of two compounds namely niclosamide (2',5-dichloro-4'-nitrosalicylanilide, ethanolamide salt) and nicotinanilide with most of the properties required for "the molluscicide molecule" (WHO 1993, DeSouza 1995). Niclosamide is currently recommended by WHO in snail control programmes (Sturrock 2001, WHO1993) in spite of its toxicity to fishes even at below molluscicidal concentration (Marking & Horgan 1967). In US, bayluscide (niclosamide) is registered as a piscicide and used more to control sea lamprey and trash fishes than snails (Schreier et al. 2000). Nicotinanilide is reported with high snail toxicity and no fish toxicity at molluscicidal concentration (DeSouza & Paulini1969). It is also reported to be very safe to fishes, non-target organisms and mammals in addition to its cercaricidal property against cercariae of schistosomes (Tang et al. 1986, Parashar et al. 1990). Even though reports are available on the toxicity of nicotinanilide against some snail vectors (Dunlop et al. 1980, Wang & Sung 1990, Cheng et al. 1991, Parashar et al. 1990, 1995), a detailed study on its molluscicidal effects is lacking. Hence, studies on the molluscicidal property of nicotinanilide as well as its intermediates like nicotinic acid hydrochloride, aniline hydrochloride and nicotinyl chloride hydrochloride were carried out against *L. luteola* snails keeping niclosamide as standard.

MATERIALS AND METHODS

Chemicals - The molluscicide nicotinanilide as a hydrochloride salt form (> 99% pure) as well as the possible intermediates of nicotinanilide such as aniline hydrochloride, nicotinic acid hydrochloride, and nicotinyl chloride hydrochloride were synthesised by the Synthetic Chemistry Division of Defence R&D Establishment, Gwalior. M/S Bayer AG, Germany supplied the niclosamide (bayluscide 70% WP) as a free sample.

Snails - The freshwater snails *L. luteola* were taken from laboratory culture maintained in enamel bowls filled with dechlorinated water at room temperature 28 ± 2^{o}C and relative humidity more than 75%. They were fed with spinach ad libitum and the water was changed twice in a

+Corresponding author. Fax: +0751-2341148. E-mail: devanathansukumaran@yahoo.co.in
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week. The egg strips containing 0-24 h and 72-96 h old eggs were used to study the ovicidal effect of molluscicides and the snails of different sizes i.e., immature (3-6 mm), young mature (9-12 mm), and adults (more than 12-mm) were used for the toxicity studies.

Toxicity studies - The toxicity of nicotinanilide and niclosamide was screened as described by WHO (1965). In the case of ovicidal action, the total number of eggs in each egg strip was counted using dissection microscope before exposure to 200 ml of test solutions kept in the petri dishes. They were exposed for 24 h and then transferred to normal water for further 24 h. Disintegration of embryos or absence of movement of the embryos was considered for calculating the per cent mortality of eggs. The toxicity of molluscicides against adult snails was carried out with ten *L. luteola* snails of each stage. They were exposed to 2 l of water in enamel bowls containing the different concentrations of the molluscicides for 24 h. After exposure, they were transferred to normal water for further 24 h and later the percent mortality of snails was calculated. All the experiments were replicated six times. The data obtained from the above studies were subjected to probit analysis (Finney 1971) to calculate LC_{50} and LC_{90} values. Similarly the LC_{50} and LC_{90} values of the intermediates of nicotinanilide were calculated against the least susceptible young mature stage of *L. luteola* snails.

Mortality pattern of the snails - The least susceptible young mature stage of *L. luteola* snails was used for this study. Ten snails in each of two groups were exposed to 2 l of tapwater containing LC_{90} concentration of nicotinanilide and niclosamide respectively. Soon after exposure, they were continuously observed at an interval of every 30 min for mortality. Extrusion of the whole head-foot region permanently outside the shell or lack of any movement of the body when touched with tip of the needle was considered to determine mortality of the snail. The duration required for the mortality of all the snails was recorded. The dead snails were removed immediately from the enamel bowl. The experiment was replicated six times. The mean duration required for the death of the snail was calculated and the data were statistically analyzed and subjected to regression curve using sigma plot.

RESULTS

The LC_{50} and LC_{90} values of nicotinanilide against eggs, immature, young mature, and adult stages of *L. luteola* are shown in Table I and mentioned in Figs 1 and 2. The LC_{90} values against the eggs of 0-24 h old and 72-96 h old are calculated as 0.76 and 0.70 ppm respectively. In the case of different growing stage of snails namely immature, young mature, and adults, the LC_{90} values are calculated as 0.67, 2.32, and 1.39 ppm respectively. The calculated values of LC_{50} and LC_{90} of niclosamide against the same snails are given in Table II and expressed in Figs 1 and 2. The values of LC_{90} of niclosamide against eggs of 0-24 h old and 72-96 h old are calculated as 0.71 and 0.38 ppm. Similarly the values of LC_{90} of niclosamide against growing stages of adults are calculated as 1.04 ppm for immature stage, 1.41 ppm for young mature stage, and 0.45 ppm against the adults.

The calculated values of LC_{50} and LC_{90} of the intermediate chemicals of nicotinanilide against the least susceptible young mature stage of *L. luteola* are given in Table III and mentioned in Fig. 3. The results show the LC_{90} value for nicotinic acid hydrochloride as 371.32 ppm, for aniline hydrochloride as 310.87 ppm and for nicotinyl chloride hydrochloride as 210.39 ppm respectively.

The overall results from the mortality pattern of the young mature stage of *L. luteola* snails exposed to LC_{90} concentrations of nicotinanilide and niclosamide are shown in Fig. 4. Complete mortality of all snails occurred within 6 to 8 h when exposed to niclosamide whereas for nicotinanilide it required between 26 and 28 h after exposure.

DISCUSSION

Ovicidal action of nicotinanilide against *L. luteola* shows the compound to be more toxic to the 72-96 h old eggs (LC_{90} value is 0.70 ppm) than the 0-24 h old eggs (LC_{90} value is 0.76 ppm). This may be due to protective

TABLE I

Toxicity of nicotinanilide against different stages of *Lymnaea luteola* snails

Stage	Regression equation	Chi Square ($p > 0.05$)	LC_{50} (ppm)	LC_{90} (ppm)
Eggs (0-24 h old)	Y = 6.53 + 2.12X	3.88	0.19 (0.16 - 0.22)	0.76 (0.57 - 1.02)
Eggs (72-96 h old)	Y = 6.56 + 1.84X	0.08	0.14 (0.12 - 0.17)	0.70 (0.47 - 1.04)
Immature (3-6 mm size)	Y = 6.76 + 2.73X	7.62	0.23 (0.20 - 0.25)	0.67 (0.54 - 0.82)
Young mature (9-12 mm size)	Y = 5.31 + 2.83X	0.55	0.77 (0.69 - 0.86)	2.32 (1.78 - 3..03)
Adults (more than 12 mm)	Y = 5.81 + 3.57X	4.75	0.59 (0.54 - 0.6)	1.39 (1.19 - 1.64)

Values in the brackets are fiducial limits 95%

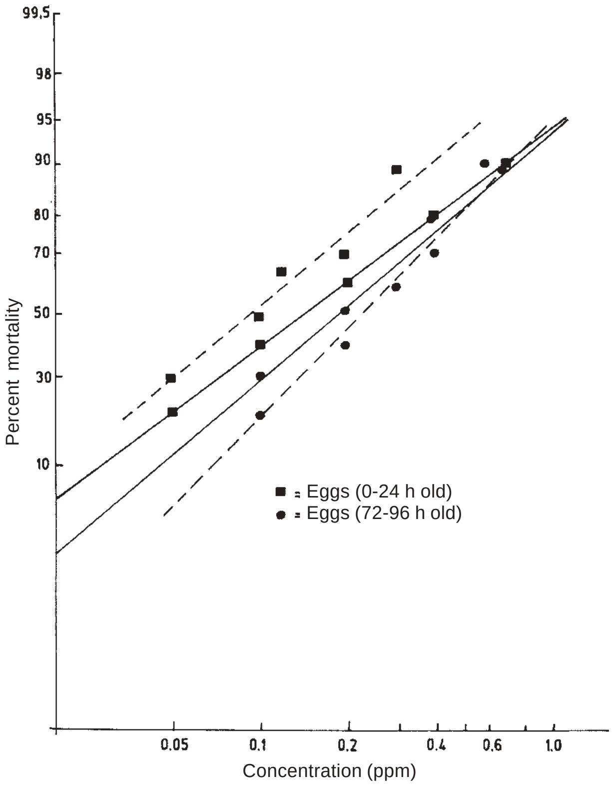

Fig. 1: concentration-mortality regression lines of two intracapsular stages of eggs of *Lymnaea luteola* exposed to nicotinanilide (——) and niclosamide(- - -).

covering of capsular jelly like material that covers and protects the freshly laid eggs from the external environment. A similar observation was also reported for nicotinanilide by Parashar et al. (1995, 1990) against *L. auricularia* (LC_{90} value against 0 to 1 day and 4 to 5 days old eggs as 1.03 mg/l and 0.57 mg/l respectively) and the eggs of *Indoplanorbis exustus* (the LC_{90} value as 0.087 mg/l). The values of LC_{50} of 4'-chloronicotinanilide against 1 to 4 days old eggs of *Biomphalaria glabrata* was reported as 10 ppm (Dunlop et al. 1980, Duncan & Brown, 1983). The ovicidal action of niclosamide against the eggs of *L. luteola* was also found to increase with the age of the eggs. Parashar et al. (1995) reported a similar trend in the ovicidal action of niclosamide against the eggs of *L. auricularia*. Copper sulphate also induced similar effects against the eggs of *Taphius glabratus* and in the embryonic developmental stage of *B. pfeifferi* (Shiff et al. 1970). The extracts of few plant molluscicides like *Euphorbia splendens*, *Phytolacca dodecandra*, *Tetrapleura tetraptera* were also reported to exhibit lower toxicity towards earlier developmental stages than adults (DeSouza et al.

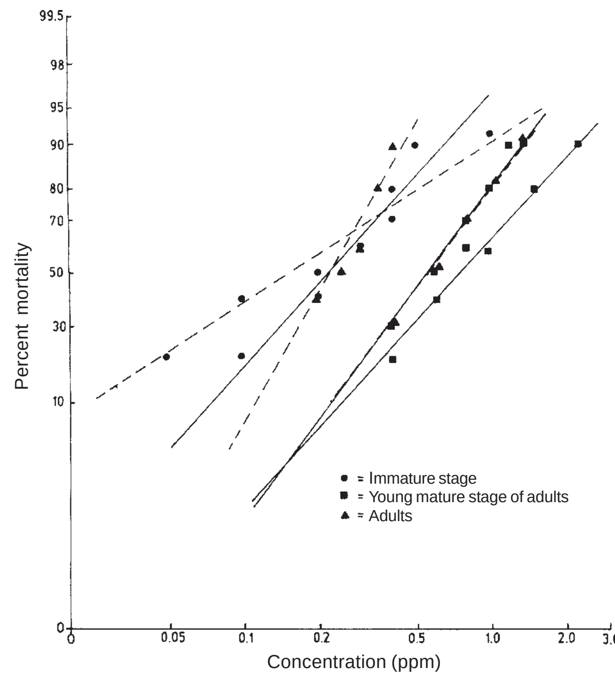

Fig. 2: concentration-mortality regression lines of different growing stages of *Lymnaea luteola* snails exposed to nicotinanilide (——) and niclosamide (- - - -).

TABLE II

Toxicity of niclosamide against different stages of *Lymnaea luteola* snails

Stage	Regression equation	Chi Square ($p > 0.05$)	LC_{50} (ppm)	LC_{90} (ppm)
Eggs (0-24 h old)	Y = 6.75 + 2.69X	3.24	0.22 (0.20 - 0.25)	0.71 (0.53 - 0.94)
Eggs (72-96 h old)	Y = 7.17 + 2.12X	3.88	0.09 (0.08 - 0.11)	0.38 (0.28 - 0.51)
Immature (3-6 mm size)	Y = 6.25 + 1.61X	5.89	0.16 (0.14 - 0.20)	1.04 (0.67 - 1.63)
Young mature (9-12 mm size)	Y = 5.83 + 3.49X	0.33	0.57 (0.52 - 0.63)	1.41 (1.14 - 1.76)
Adults (more than 12 mm)	Y = 8.02 + 4.88X	6.36	0.24 (0.22 - 0.25)	0.45 (0.40 - 0.51)

Values in the brackets are fiducial limits 95%

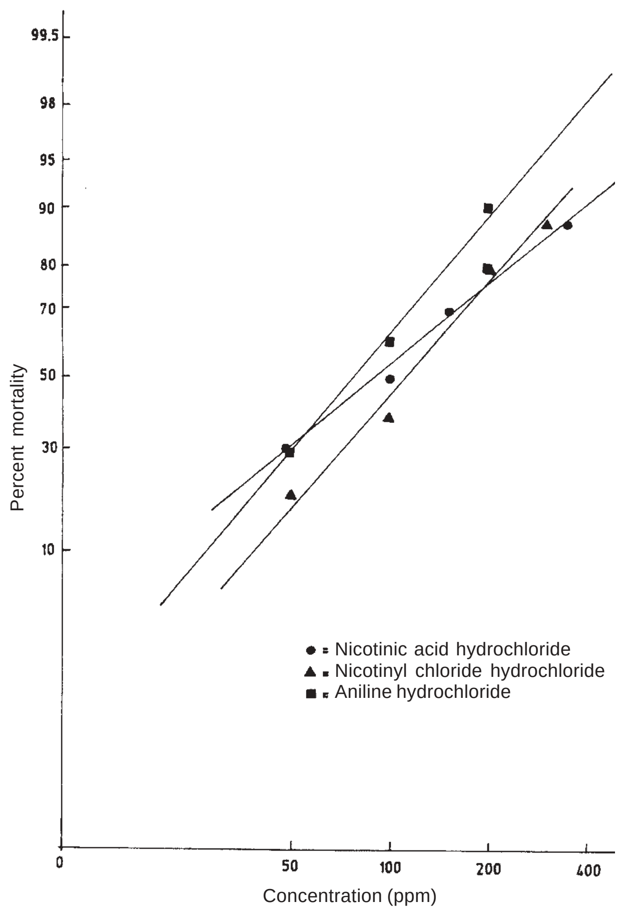

Fig. 3: concentration-mortality regression lines of intermediate compounds of nicotinanilide against young mature stage of adults of *Lymnaea luteola* snails.

1987, Schall et al. 1988, Adewunmi,1991). On the contrary, the jelly like egg protectant covering ova in *L. natalensis* was reported to be easily penetrated by niclosamide and produced 100% mortality (Gillet & Bruaux 1962). Zhang and Guo (1992) reported that the eggs of *Oncomelania* snails exposed to bromoacetamide showed the deformation in the earlier stages but not in the later stages. Sukumaran et al. (1994, 1995, 2002) also reported the n-butanol extracts of some plant molluscicides like *Sapindus trifoliatus, Agave americana, Balanites aegyptica, Jatropha gossypifolia*, and *Vaccaria pyramidata* as toxic against freshly laid eggs of *L. luteola*.

Studies on the toxicity of both nicotinanilide and niclosamide against different growing stage of *L. luteola* snails indicated both the compounds as more toxic to both immature and adult stages of the snails than the young mature stage. Parashar et al. (1990) also reported the toxicity of nicotinanilide against *I. exustus* snails and indicated the young adult stage as least susceptible. The reason may be that the young mature adult stage of the *L. luteola* snails is highly active and reproductive and hence more tolerant to the different environmental stress (changes in pH, hardness of water, and different pollutants including pesticides and molluscicides) present in the aquatic medium. In this study, the toxicity of niclosamide against young mature stage of *L. luteola* was found to be 1.64 times higher than nicotinanilide. Niclosamide is also reported as more toxic than nicotinanilide against young mature stage of *L. auricularia* (Parashar et al. 1995).

Toxicity of intermediates of nicotinanilide against the young mature stages of *L. luteola* shows them as very poor molluscicides as illustrated by their higher LC_{90} values. The acidic moiety of niclosamide namely 2'5-dichloro 4'-aminosalicylanilide is reported to loose all its molluscicidal properties and found ineffective in killing snails (Struff 1964, Struff & Gonnert 1967).

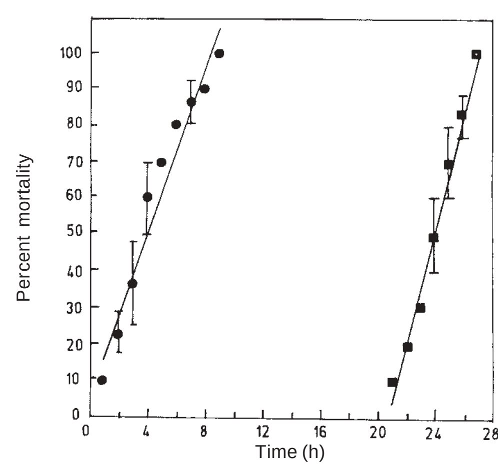

Fig. 4: mortality pattern of the young mature stage of adults of *Lymnaea luteola* snails exposed to LC_{90} concentration of nicotinanilide (■) and niclosamide (●).

TABLE III

Toxicity of intermediates of nicotinanilide against young mature stages of *Lymnaea luteola* snails

Compound	Regression equation	Chi Square (p > 0.05)	LC_{50} (ppm)	LC_{90} (ppm)
Nicotinic acid hydrochloride	Y = 0.58 + 2.25X	1.01	89.77 (76.39 - 105.50)	371.32 (247.45 - 557.20)
Aniline hydrochloride	Y = 0.71 + 2.80X	2.47	108.46 (95.48 - 123.21)	310.87 (232.52 - 415.62)
Nicotinyl chloride hydrochloride	Y = 0.58 + 2.95X	0.59	77.55 (68.19 - 88.21)	210.39 (167.02 - 265.03)

Values in the brackets are fiducial limits 95%

The studies on the mortality pattern of *L. luteola* snails show that niclosamide requires less time for complete mortality of the snails than nicotinanilide. The rapid action of niclosamide in killing snails is due to its toxic effect on the respiratory function of the snails by acting as uncoupler of oxidative phosphorylation at the mitochondrial level (White House 1964, Andrews et al. 1983). Highest mortality of the *L. luteola* snails in this study was observed during the period between 6 and 8 h after exposure. In the case of *L. cailliaudi* the complete inhibition of oxygen uptake was observed after 6 h when the snails were exposed to 2 mg/l of niclosamide (Elgindy & Mohamad 1976). In the case of nicotinanilide, mortality of the snails started only after 21 h of exposure, the slow molluscicidal action may require its accumulation into the body of the snail. Daffalla (1978) exposed *B. glabrata* snails to nicotinanilide and studied the thin layer chromatography of the chloroform extract of pseudobranch and reported only the presence of parent compound i.e., nicotinanilide but no metabolite.

From the point of view of safety to non-target organisms, fishes, and mammals, nicotinanilide may be regarded as a better option even though the LC_{90} value is 1.6 times higher than niclosamide. Hence, nicotinanilide can be recommended for selective killing of snails in schistosomiasis control programmes.

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