American-Eurasian Journal of Toxicological Sciences 3 (2): 70-74, 2011
ISSN 2079-2050

Molluscicidal Effect of Freeze-dried Cow Urine of Indian Cow Breeds against *Lymnaea acuminata*

Shiv Kumar, Dinesh Kumar Singh and Vinay Kumar Singh

Department of Zoology, DDU Gorakhpur University,
Gorakhpur, 273 009, UP, India

Abstract: Molluscicidal activity of different formulations of freeze-dried cow urine (FCU) of different Indian breeds such as, *Shahiwal, Geer* and *Tharparkar* was studied against the snail *Lymnaea acuminata,* which is the intermediate host of liver fluke *Fasciola gigantica*. The toxicity of cow urine of different breeds kept for 15 days in sunlight (8h/day) or in ambient laboratory condition, as well as boiled urine samples were tested against the *L. acuminata*. Freeze-dried cow urine (FCU) of different Indian breeds; *Shahiwal, Geer* and *Tharparkar* were toxic against snail *Lymnaea acuminata.* Toxicity of *Thaparkar* breed urine kept for 15 days in sunlight or in laboratory condition was higher than *Shahiwal* and *Geer* sunlight laboratory. Toxicity of all samples was time and concentration dependent. Toxicity of *Thaparkar* breed urine kept for 15 days in sunlight (24h LC_{50}- 604.85mg/l) and laboratory condition (24h LC_{50}- 519.71mg/l) was higher than *Shahiwal* (24h LC_{50}- sunlight, 650.70mg/l and laboratory conditions, 666.98 mg/l) and *Geer* (24h LC_{50}- sunlight, 619.01mg/l and in laboratory conditions, 674.33 mg/l). Molluscicidal activity was also observed in boiled cow urine. Boiled urine of Geer (24h LC_{50}- 519.75mg/l) and *Shahiwal,* (24h LC_{50}- 519.88mg/l) was more toxic than *Tharparkar* (24h LC_{50}- 664.17mg/l). Cow urine formulation of different breeds may be used as potent molluscicides.

Key words: Freeze-dried cow urine (FCU) · *Lymnaea acuminata* · Fascioliasis · Molluscicide

INTRODUCTION

Fascioliasis is one of the most debilitating zoonotic diseases [1]. This disease ranks as a major cause of morbidity and mortality both in man and live-stock and contribute to socioeconomic problem [2]. Human infection has been reported in 51 different countries from 5 continents. In human the disease is characterized by hypereosinophilia, abdominal pain and pancreatitis [3]. The freshwater snail *Lymnaea* (Radix) *acuminata* (Lamarck) is the intermediate host of *Fasciola gigantica*, which causes endemic fascioliasis in cattle population of northern part of India [4]. The problem of fascioliasis can be eliminated by killing the intermediate host of the fluke. Several attempts have been made to reduce the incidence of fascioliasis by using synthetic and plant molluscicides [5, 6]. Majority of synthetic molluscicides are environmentally unsafe. Use of medicinal formulations containing cow urine, buttermilk and cow ghee mixed with herbs is very common in Indian Ayurvedic system of treatments [7]. It has been reported that use of different preparations of cow urine and dung can prevent viral, bacterial and fungal diseases in plants [8]. Tripathi et al., [9] and Kelkuda et al., [10] have reported that different cow urine preparations have sufficient molluscicidal, pesticidal and antihelmintic activity. Cow urine has antioxidant and antimicrobial activity [11, 12]. The molluscicidal/ pesticidal property of cow urine will be advantageous as it does not have the poisonous hazards of synthetic pesticides. However, scientific data are lacking on the pharmacology and pharmaceutical properties of cow urine. Chemistry of inherent bioactive compounds present in cow urine is almost lacking. The present study describes the molluscicidal activity of urine of different Indian breeds such as, *Shahiwal, Geer* and *Tharparkar* against the snail *Lymnaea acuminata.*

MATERIALS AND METHODS

Collection of Cow Urine: Urine of *Shahiwal, Geer* and *Tharparkar* breeds were collected in sterilized bottles, from green grass grazing 3-5 year old healthy cows.

Corresponding Author: Vinay Kumar Singh, Department of Zoology, DDU Gorakhpur University, Gorakhpur, 273 009, UP, India. Mob.: +91-9415855488, E-mail: vksingh_gpu@yahoo.co.in.

No other food materials were given to the experimental animals. Different formulations of cow urine were tested against the snail *L. acuminata*.

Preparation of Freeze-dried Cow Urine (FCU): Freeze-dried fractions of the urine were used in w/v treatment as following:

- Freeze-dried powder of fresh cow urine of *Shahiwal*; *Geer* and *Tharparkar* (1ml urine = 20-30 mg freeze-dried), respectively.
- Freeze-dried powder of *Shahiwal* (1ml urine = 25-30 mg freeze-dried); *Geer* (1ml urine = 20-30 mg freeze-dried); *Tharparkar* (1ml urine = 25-35 mg freeze-dried) urine kept for 15 days under ambient laboratory condition.
- Freeze-dried powder of *Shahiwal* (1ml- urine = 25-30 mg freeze-dried); *Geer* (1ml urine = 20-30 mg freeze-dried); *Tharparkar* (1ml urine = 25-30 mg freeze-dried) urine kept for 15 days in 8h/day sunlight.
- Freeze-dried powder of boiled urine of *Shahiwal* (1ml urine = 15-25 mg freeze-dried); *Geer* (1ml urine = 20-30mg freeze-dried); *Tharparkar* (1ml urine = 15-25 mg freeze-dried).

Snails: Adult *L. acuminata* (2.60±0.30cm in length) were collected locally from ponds, pools, lakes and low-lying submerged areas located almost adjacent to DDU Gorakhpur University campus. The collected snails were acclimatized in dechlorinated tap water for 72 h. Ten snails were kept in a glass aquarium containing 3 liter of dechlorinated tap water at 22°C to 24°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2 mg/l, 5.2-6.3 mg/l and 102.0-105.0 mg/l, respectively. Dead snails were removed immediately from the aquaria to avoid any contamination.

Toxicity Experiment: Toxicity experiment was performed by the method of Singh and Agarwal [13]. The snails were exposed to the different concentrations of the FCU of different Indian breeds in water aquaria. Six aquaria were set for each concentration. Mortality of the snail was recorded at 24h intervals to a total of 96h. Snail mortality was established by contraction of the body within the shell and absence of response to a needle probe.

Statistical Analysis: The LC values, lower and upper confidence limits (LCL, UCL), slope value, t-ratio, g-values and heterogeneity factors were calculated by using POLO computer software of Robertson et al. [14]. The regression coefficient exposure time and different values of LC_{50} were determined by the method of Sokal and Rohlf [15].

RESULTS AND DISCUSSION

The fresh urine samples of different cow breeds were pale yellow in colour. Urine kept for 15 days in sunlight turned from pale yellow to dark, while under laboratory conditions the colour was light brown after 15 days. There was no change in colour of boiled urine. The pH of fresh cow urine of *Shahiwal, Geer* and *Tharparkar* breeds were 8.2, 8.9 and 8.15, respectively. While the pH of cow urine kept for 15 days in sunlight were 9.4 for *Shahiwal,* 9.58 for *Geer* and 9.12 for *Tharparkar*. In ambient laboratory conditions, the pH values of urine samples raised (*Shahiwal*- pH-9.75, *Geer*-9.20 and *Tharparkar*-9.32). pH of boiled urine of (*Shahiwal*-8.5), (*Geer*-9.39) and (*Tharparkar*-8.79) were also higher than fresh urine.

Furthermore, the conductivity of fresh cow urine of *Shahiwal, Geer* and *Tharparkar* breeds were 128.6, 93.00 and 120.53 ntu, respectively. Cow urine kept for 15 days in sunlight (*Shahiwal*- 145.5, *Geer*-95.00 and *Tharparkar*-134.53 ntu) and in ambient laboratory conditions (*Shahiwal*- 192.5, *Geer*-97.7 and *Tharparkar*-148.49 ntu) showed higher conductivity. Boiled urine conductivity of *Shahiwal* (199.7 ntu), *Geer* (80.9 ntu) and *Tharparkar* (172.5 ntu) were also higher than fresh urine except *Geer* breed.

The 24h LC_{50} of FCU of *Tharparkar* urine kept for 15 days in sunlight (604.85 mg/l) and in ambient laboratory condition (519.71mg/l) were lower than LC_{50} values of *Shahiwal* (sunlight- 650.70 mg/l and laboratory conditions- 666.98) and *Geer* (sunlight- 619.01mg/l and laboratory conditions- 674.33 mg/l). The 24h LC_{50} of boiled FCU of *Geer* (519.75 mg/l) and *Shahiwal* (519.88 mg/l) were lower than *Tharparkar* (664.17 mg/l). 96h LC_{50} of FCU of *Tharparkar* urine kept for 15 days in sunlight (288.40 mg/l) and in ambient laboratory conditions (243.91mg/l) were lower than LC_{50} values of *Shahiwal* (sunlight- 333.78 mg/l and laboratory conditions- 360.39) and *Geer* (sunlight- 356.30 mg/l and laboratory conditions- 415.41 mg/l). The 96h LC_{50} of boiled FCU of *Geer* (228.88 mg/l) was lower than *Shahiwal* (300.49 mg/l) and *Tharparkar* (319.17 mg/l).

The slope values given in Tables 1-3 were steep. Separate estimation of LC based on each of the six replicates was found to be within 95% confidence limits of LC_{50}. The t- ratio was greater than 1.96 and the heterogeneity factor is less than 1.0. The g- value was less than 0.5 at all probability levels (90, 95 and 99).

Table 1: Toxicity of freeze-dried cow urine powders (FCU) of different breeds kept for 15 days in sunlight against *L. acuminata*

Exposure period	Treatment	LC_{50} mg/l (w/v)	Limits LCL - UCL	Slope value	t-ratio	g-value	heterogeneity
24h	*Shahiwal* cow urine	650.70	555.95-914.91	4.00±0.85	4.66	0.17	0.29
	Geer cow urine	619.01	558.58-744.98	5.06±0.94	5.37	0.23	0.25
	Tharparkar cow urine	604.85	500.87-918.02	3.22±0.70	4.55	0.18	0.26
48h	*Shahiwal* cow urine	550.00	483.55-701.00	3.65±0.74	4.90	0.16	0.24
	Geer cow urine	538.54	484.79-642.57	3.82±0.79	4.81	0.18	0.22
	Tharparkar cow urine	604.85	500.87-918.02	3.22±0.70	4.55	0.18	0.26
72h	*Shahiwal* cow urine	458.51	400.01-576.40	2.76±0.66	4.13	0.22	0.20
	Geer cow urine	446.27	397.33-505.24	3.46±0.75	4.58	0.32	0.15
	Tharparkar cow urine	403.11	346.78-512.06	2.51±0.57	4.35	0.20	0.25
96h	*Shahiwal* cow urine	333.78	293.33-368.27	3.92±0.68	5.71	0.18	0.60
	Geer cow urine	360.39	315.57-394.34	4.49±0.79	5.68	0.17	0.22
	Tharparkar cow urine	288.40	245.64-326.23	3.09±0.58	5.33	0.13	0.47

Six batches of ten snails were exposed to different concentrations of FCU. Mortality was recorded at every 24h for 4 days. Concentrations given are the final concentration (w/v) in glass aquarium water. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. Testing significant ts of regression coefficient of different FCU, *Shahiwal* cow urine -3.97^{+}; *Geer* cow urine -56.20^{+}; *Tharparkar* cow urine -8.09^{+}.
+: linear regression between x and y; ++: non – linear regression between log x and log y.

Table 2: Toxicity of freeze-dried cow urine powders (FCU) of different breeds urine kept for 15 days in ambient laboratory condition against *L. acuminata*

Exposure period	Treatment	LC_{50} mg/l (w/v)	Limits LCL - UCL	Slope value	t-ratio	g-value	heterogeneity
24h	*Shahiwal* cow urine	666.98	593.71-838.70	5.24±1.03	5.09	0.14	0.35
	Geer cow urine	674.33	604.02-841.57	4.62±0.97	4.74	0.18	0.23
	Tharparkar cow urine	519.71	450.19-680.43	3.49±0.69	5.06	0.15	0.27
48h	*Shahiwal* cow urine	600.67	527.20-789.38	3.51±0.80	4.36	0.20	0.17
	Geer cow urine	603.66	546.32-722.37	4.13±0.88	4.65	0.22	0.17
	Tharparkar cow urine	443.16	384.90-564.50	2.87±0.60	4.75	0.17	0.14
72h	*Shahiwal* cow urine	462.50	394.83-572.02	2.52±0.73	3.41	0.32	0.22
	Geer cow urine	507.12	460.31-566.16	4.04±0.85	4.74	0.16	0.15
	Tharparkar cow urine	331.86	282.08-389.82	2.57±0.57	4.51	0.18	0.13
96h	*Shahiwal* cow urine	356.30	314.09-388.42	4.77±0.80	5.90	0.11	0.68
	Geer cow urine	415.41	369.77-450.03	4.99±0.88	5.65	0.17	0.25
	Tharparkar cow urine	243.91	191.31-281.33	2.89±0.58	4.94	0.15	0.43

Six batches of ten snails were exposed to different concentrations of FCU. Mortality was recorded at every 24h. Concentrations given are the final concentration (w/v) in glass aquarium water. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. Testing significant ts of regression coefficient of different FCU, *Shahiwal* cow urine -11.05^{+}; *Geer* cow urine -21.98^{+}; *Tharparkar* cow urine -20.92^{+}.
+: linear regression between x and y; ++: non – linear regression between log x and log y.

Table 3: Toxicity of freeze-dried cow urine powders (FCU) obtained from the boiled urine of different breeds against *L. acuminata*

Exposure period	Treatment	LC_{50} mg/l (w/v)	Limits LCL - UCL	Slope value	t-ratio	g-value	heterogeneity
24h	*Shahiwal* cow urine	519.88	475.15-596.73	5.11±0.83	6.11	0.10	0.24
	Geer cow urine	519.75	450.08-679.83	3.48±0.68	5.10	0.18	0.22
	Tharparkar cow urine	664.17	557.06-998.51	3.56±0.18	4.38	0.19	0.27
48h	*Shahiwal* cow urine	466.80	422.73-538.18	3.99±0.71	5.60	0.12	0.16
	Geer cow urine	418.19	375.39-486.78	3.71±0.63	5.83	0.22	0.29
	Tharparkar cow urine	550.77	468.87-795.79	2.77±0.69	3.99	0.24	0.21
72h	*Shahiwal* cow urine	378.47	324.12-426.22	3.37±0.66	5.04	0.15	0.20
	Geer cow urine	311.67	259.28-364.20	2.50±0.56	4.41	0.17	0.15
	Tharparkar cow urine	384.36	336.67-438.34	3.13±0.66	4.70	0.17	0.28
96h	*Shahiwal* cow urine	300.49	260.27-331.34	4.30±0.71	6.04	0.10	0.38
	Geer cow urine	228.98	177.53-264.68	3.05±0.59	5.09	0.16	0.56
	Tharparkar cow urine	309.17	268.28-341.12	4.14±0.70	5.90	0.11	0.53

Six batches of ten snails were exposed to different concentrations of FCU. Mortality was recorded at every 24h. Concentrations given are the final concentration (w/v) in glass aquarium water. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments. Testing significant ts of regression coefficient of different FCU, *Shahiwal* cow urine -14.66^{+}; *Geer* cow urine -27.09^{+}; *Tharparkar* cow urine -18.31^{+}.
+: linear regression between x and y; ++: non – linear regression between log x and log y.

The current data clearly demonstrate that cow urine of different Indian breeds kept for 15 days in sunlight, ambient laboratory conditions and boiled urine are potent molluscicide. Freeze-dried powder of fresh urine is not toxic against *L. acuminata*. It has been reported by various scientists the potent antifungal and antibacterial activities of cow urine and their distillate [10, 12, 16]. Probably, fermentation of urine resulted in toxic components within 10 days. It also seems that the urea is a major element found in urine and is the end product of protein metabolism. It is strong antibacterial agent. Similarly, uric acid is also having strong antibacterial properties. Urine kept for 10 days was less toxic than that 15 days, which indicates that concentration of the molluscicidal component was maximum in 15 days old urine. FCU of *Tharparkar* urine kept for 15 days in sunlight or in ambient laboratory conditions were more toxic than both *Shahiwal* and *Geer* cow urine, while the boiled *Geer* urine was more toxic than the other breeds. Recently, Tripathi et al., [17] reported that cow urine kept in sunlight for 15 days singly and their binary combination with other herbal molluscicides significantly reduced the fecundity of snail *L. acuminata*. Cow urine behaves as bio-enhancer in pharmaceutical composition, as it enhances the antimicrobial effect of antibiotics [16]. It has been effectively used for control of tsetse fly. Adane and Gautam [18] have been reported both insecticidal and fungicidal activity of fermented cow urine. The odour of cow urine is a strong attractant for *Glossina pallidipes* and also catches 60% of the vector of *Trypanosoma* [19-21].

It is evident from the steep slope value that a small increase in the concentration of different FCU of different breeds of Indian cow urine caused higher snail mortality. A t-ratio value greater than 1.96 indicates that the regression is significant. Heterogeneity factor values less than 1.0 denote that in the replicate tests of random sample the concentration response curve would fall within the 95% confidence limits and thus the model fits the data adequately. The index of significance of the potency estimation g indicates that the value of the mean is within the limit at all probability levels (90, 95 and 99) since it is less than 0.5.

Cow urine formulation of different breeds may be used as potent molluscicides. Its molluscicidal activity is the sign that it can be evaluated against harmful pests in agricultural field. This technique of pest control will be an indigenous biotechnological tool which can be easily used by the farmers in our country. Its attraction was and is largely based on the philosophy of self-reliance and safe environmental use.

REFERENCES

1. World Health Organization, 2007. Report of the WHO Informal Meeting on Use of Triclabendazole in Fascioliasis Control, WHO Headquarters, Geneva, Switzerland. 17-18 October. 2007 WHO/CDS/NTD/PCT/2007.1.
2. Mas-Coma, S., M.D. Bargues and M.A. Valero, 2005. Fascioliasis and other plant-borne tremadode zoonoses. Intl. J. Parasitol., 35: 1255-1278.
3. Saba, R., M. Korkmaz, D. Inan, L. Mamikoglu, O. Turhan, F. Gunseren, C. Cevikol and A. Kabaalioglu, 2004. Human fascioliasis. Clin. Microbiol. Infection, 10(5): 385-387.
4. Singh, O. and R.A. Agarwal, 1981. Toxicity of certain pesticides to two economic species of snail in northern India. J. Econom. Entomol., 74: 568-571.
5. Singh, A., D.K. Singh, T.N. Misra and R.A. Agarwal, 1996. Molluscicides of plant origin. Biological, Agriculturte and Horticulture, 13: 205-252.
6. Kumar, P. and D.K. Singh, 2006. Molluscicidal activity of *Ferula asafetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. Chemosphere, 63: 1568-1574.
7. Shastri, A.D., 1998. Shushrut Samhita. 11th ed Chaukhanba Sanskrit Prakashan, Varanasi, India.
8. Sridhar, S., S. Arumugasamy, H. Saraswathi and K. Vijayalakshmi, 2002. Organic Vegetable Gardening: First Edition. Centre for Indian Knowledge Systems, Chennai, India.
9. Tripathi, R., V.K. Singh and D.K. Singh, 2006. Freeze–dried powder of cow urine reduces the viability of the snail *Lymnaea acuminata*. J. Pest Sci., 79: 143-148.
10. Kelkuda, P.T.R., B.C. Nishanth, P.S.V. Kumar, D. Kamal, M. Sandeep and H.K. Meghraj, 2010. Cow Urine Concentrate: A potent agent with antimicrobial and anthelmintic activity. Drug Invention Today, 3(5): 1025-1027.
11. Jarald, E., S. Edwin, V. Tiwari, R. Garg and E. Toppo, 2008. Antioxidant and antimicrobial activities of cow urine. Global J. Pharmacol., 2(2): 20-22.
12. Sathasiyam, A., M. Muthuselyam and R. Rajendran, 2010. Antimicrobial activities of cow urine distillate against some clinical pathogens. Global J. Pharmacol., 4(1): 41-44.
13. Singh, D.K. and R.A. Agarwal, 1984. Correlation of the anticholinesterase and molluscicidal activity of the latex of the *Euphorbia royleana* Bioss on *Lymnaea acuminata*. J. Natural Products, 47: 702-705.

14. Robertson, J.L., R.M. Russell, H.K. Preisler and N.E. Savin, 2007. Bioassay with arthropods POLO. Computer programme for analysis data 2nd Eds: (Taylar and Francis, CRC Press) pp: 1-224.
15. Sokal, R.R. and F.J. Rohlf, 1973. Introduction of biostatistics, W.H. Freeman, San Francisco, pp: 185-207.
16. Khanuja, S.P.S. *et al.,* 2002. Pharmaceutical composition containing cow urine distillate and an antibiotic. US Patent 6410059.
17. Tripathi, R., P. Kumar, V.K. Singh and D.K. Singh, 2010. Cow urine and their formulations with plant molluscicide against the reproduction of vector snail *Lymnaea acuminata*. Drug Invention Today, 2(8): 376-380.
18. Adane, T. and R.D. Gautam, 2003. Traditional pest management practices and lesser exploited natural products in Ethopia and India; Appraisal and revalidation. Indian J. Traditional Knowledge, 2: 189-201.
19. Oloo, F.P., 1992. Comparison of baited and unbaited NGU and biconical traps for *Glossina pallidipes* and *G. longipennis* and the combination of odour combinations in Nguruman, Kaijado District, Kenya. In: proceedings of a seminar organized by the International atomic energy, pp: 233-241.
20. Opiyo, E.A. and J.K. Omuse, 1992. Experience with odour baited insecticide impregnated targets for control of tsetse flies in Kenya. In: proceedings of a seminar organized by the International atomic energy, pp: 191-192.
21. Tikubet, G., 1992. Integrated tsetse management in south western Ethiopia. In: proceedings of a seminar organized by the International atomic energy, pp: 207-208.