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DIFFERENT PREPARATIONS OF *Solanum surattense* AND THEIR EVALUATION AGAINST FECUNDITY, HATCHABILITY, AND SURVIVAL OF THE SNAIL *Lymnaea acuminata*

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ABSTRACT

Snail is an intermediate host of fascioliosis caused by *Fasciola*. These parasitic diseases are caused by trematode species of *Fasciola hepatica* and *F. gigantica* in cattle and humans. The carrier of the *Fasciola* is a freshwater host snail *Lymnaea acuminata*. The control of the host snail is maybe a major technique for reducing of fascioliosis. Synthetic molluscicide causes an adverse effect in the environment as well as on non-target organisms. Direct killing of the host snails is maybe ecological imbalances. The present studies were designed for the evaluation of anti-reproductive/molluscicidal properties of medicinal plant *Solanum surattense* on fecundity, hatchability, and survival of the host snail *L. acuminata*. The effect of sub-lethal (40 and 80% of 24h LC_{50}) concentration of leaf powder, different organic extract, and column purified of *S. surattense* were studied on the reproduction (fecundity, hatchability, and survival) of snail *L. acuminata*. It was observed that the different preparations of *S. surattense* significantly reduced the fecundity, hatchability and survival of the young snails *L. acuminata*. Maximum reduction in fecundity, and hatchability after 24h was noted in column purified treated snail. No survival was observed after 24h. Significant recovery in the reproductive capacity of treated snails was noted in the withdrawal group of the snail. The percent fecundity increased concerning control after withdrawal from 72h treated of the host snails.

KEYWORDS: *Solanum surattense*; *Lymnaea acuminata*; Fecundity; Hatchability; Survival of Snail

Fasciola hepatica and *F. gigantica* is a causative agent of the fascioliosis (Mas-Coma *et al.,* 2007; Hacariz *et al.,* 2014). Fascioliosis is a worldwide parasitic zoonotic disease in cattle and humans (Mas-Coma *et al.,* 2014; Cwiklinski *et al.,* 2016). Worldwide it is estimated that around 2.4 to 17 million people are infected through fascioliasis (Fairweather *et al.,* 2020). In India, this disease is mainly caused by the species of *F. gigantica* (Dalton, 1999). The life cycle of *Fasciola* is very complex which is completed in between intermediate host snails and definitive host mammals including humans (Carvedo and Cabad, 2020). Fasciola lives in the liver of cattle, which have significant importance on developments, growth rate, and productivity of livestock which have economically significant (Eshetu *et al.,* 2017). In the eastern part of Uttar Pradesh, (India), snail *L. acuminata* is an intermediate host of *F. gigantica*, which transmitted endemic of fascioliosis among cattle populations (Singh and Agarwal, 1981; Kumar and Singh, 2006; Kumar *et al.,* 2011; Kumar *et al.,* 2012; Kumar *et al.,* 2013a; 2013b; Kumar and Singh, 2014; Kumar *et al.,* 2016; Kumar *et al.,* 2018a; 2018b; Kumar *et al.,* 2020). Some species of molluscs are also causing greater economical loss by damaging crops and ornamental plants in the gardens (Kumar, 2020). Therefore, the control of snail population below threshold level, thereby breaking the life cycle of *Fasciola* (Kumar and Singh, 2006; Kumar *et al.,* 2009; Kumar *et al.,* 2018a; Kumar, 2021a) and reduce the incidence of fascioliosis and control the economic loss. The control of the snail population below the threshold level by using anti-reproductive/molluscicides is a well-recognized method for control of fascioliosis. Synthetic anti-reproductive/molluscicides are not safer for the environment (Agarwal and Singh, 1988). Therefore, the controls of the snail population by use of plant products are effective for several life stages of the harmful snails and safer for the non-target organisms as well as the environment (Singh *et al.,* 1996).

Phytochemicals are becoming an alternative source of synthetic anti-reproductive/ molluscicides because they are more acceptable, and safer for the environment and non-target aquatic organisms, as well as biodegradable (Marston and Hostettmann, 1985; Kumar, 2021a). The medicinal plant *Solanum surattense* is widely distributed throughout the tropical and subtropical parts of South East Asia (Khare, 2007). This plant is traditionally used for the treatment of fever, leprosy, cough, asthma, dropsy, dysmenorrheal hypertension, cardiac disorder, epilepsy, and depression (Vaidyaratnam, 1994; Khan and Khan, 2019; Kumar, 2021b). *S. surattense* have antioxidant, antipyretic (Muthalik *et al.,*

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2003), antiulcer, antimicrobial, anti-inflammatory, and anthelmintic activity (Pawar and Maheshwari, 2003). The pharmacological study of *S. surattense* evaluated for analgesic (Amirtharaj *et al.,* 2015), antibacterial, antinociceptive, antidiabetic, antioxidant, antifungal and larvicidal (Ramar and Nandagopalam, 2011) activities. The present study aims is to evaluate the different preparations of *S. surattense* against fecundity, hatchability, and survival of the freshwater host snail *L. acuminata.*

MATERIALS AND METHODS

Collection of Experimental Snails

Adult snail *L. acuminata* (2.62±0.33 cm in length) were collected from ponds, pools and low-lying submerged fields from Muhammadabad Gohna, Mau, Uttar Pradesh, India. The snails were acclimatized for 48 hours in dechlorinated tap water at 26±4^{0}C in lab conditions.

Preparation of Plant Products

The leaves of *S. surattense* were collected from the college campus. These leaves were washes with fresh water and dried in sunlight for 3 to 5 days and pulverized in the grinder machine for crude powders thus obtained, were then sieved with the help of a fine sterilized mesh cloth. This fine crude powder was then used for the preparation of organic extracts, column purified fraction and all these preparations are used for evaluation against fecundity, hatchability, and survival of the snail *L. acuminata*.

Organic Extractions

Five-gram leaf powders of *S. surattense* were extracted with 500 ml of organic solvents (98% ether, 99.7% chloroform, 98% methanol, 98% acetone, and 95% ethanol) for 24h at room temperature. Separately each preparation was filtered through Whatman No-1 filter paper and the filtered extracts were subsequently evaporated under a vacuum machine (Jaiswal and Singh, 2008). The extracted leaf of *S. surattense* yielded 250 mg ether, 215 mg chloroform, 230 mg methanol, 240 mg acetone, and 235 mg ethanol extracts. These extracts separately were used for the evaluation against fecundity, hatchability, and survival of the snails *L. acuminata*.

Preparation of Column Fractions

One thousand milliliters of ethanol was used for column extraction of dried leaf powder of *S. surattense* were subjected to silica gel (60-120 mesh, Qualigens Glass) chromatography through a 5 × 45 cm column. Five hundred milliliter fractions eluted with ethanol were collected and it was evaporated under a vacuum machine and the remaining solids column extract obtained was used for the evaluation against fecundity, hatchability, and survival of the host snail *L. acuminata*.

Treatment of Snails with Sub-Lethal Dose

Experimental snails were treated with sub-lethal (40% and 80% of 24h LC_{50}) concentrations of dried leaf powder of *S. surattense,* different organic extracts, and column purified fractions (Kumar, 2021c) on the reproduction was studied by the method of Kumar *et al.,* (2013b). Groups of 20 snails in 3L tap water were treated with sub-lethal concentrations (40% and 80% of 24h LC_{50}) leaf powder of *S. surattense,* of different organic extracts (ether, chloroform, methanol, acetone, and ethanol), and column purified fractions for the fecundity, hatchability and survival of the host snails.

Experimental Essay (Fecundity, Hatchability, and Survivability of the Snail)

The experiments fecundity, hatchability, and survivability were performed by the method of Kumar *et al.,* (2013b). The total number of eggs laid by snails treated with sub-lethal (40% and 80% of 24h LC_{50}) concentration of plant-derived anti-reproductive/molluscicides and control group of snails were counted every 24h for 96h. Since it is difficult to detect the mother snails for the particular spawn, capsules containing eggs from each treated and control group were incubated at 31°C in covered Petri dishes containing the same concentration as those given to adult snails. The development of snail embryos at regular intervals was observed under a binocular light microscope until they hatched. A dead embryo lacks embryonic movements and becomes opaque. Dead embryos were removed to avoid any contamination. Young snails were immediately transferred to freshwater and their survival was observed up to 72h after hatching. Each experiment was replicated six times. In a withdrawal experiment, the snail was transferred to freshwater after 96h of exposure to the above-mentioned treatment, and their fecundity was observed for the next 72h.

Statistical Calculations

Each experiment was replicated at least 6 times for statistical calculations. Values were expressed as Mean ± SE. Students t-test was applied to determine the significant ($p<0.05$) difference between sub-lethal treated and control group of the experiment. The product-moment correlation coefficient was applied in between exposure time and different values of fecundity/ survival of hatched snails (Sokal and Rohlf, 1973).

RESULTS

In control groups, each snail laid egg per day and 20 snails laid 180-190 eggs/day. There was a significant ($p<0.05$) reduction in the fecundity of snail *L. acuminata* treated with a sub-lethal concentration of 40 and 80% of 24h LC_{50} of dried leaf powder, ether extract, chloroform extract, methanol extract, acetone extract, ethanol extract and column purified fractions was exposed (Table-1). No egg lying after 72h was observed in snails treated with 40 and 80% of 24h LC_{50} of column purified fractions of dried leaf powder of *S. surattense*. The hatching period was prolonged in the treated group (11-17 days) to the control group (8-12 days) (Table-2). Withdrawal of snail after 96h treated snail for the next 72h in freshwater caused a significant ($p<0.05$) recovery in the fecundity of snails to their corresponding treatment (Table-1).

After 72h no survival of young snail was noted in treated with 80% of 24h LC_{50} of ethanol extract, whereas after 24h no survival was observed in young snail treated with 40% or 80% of 24h LC_{50} of column purified fractions (Table-2). There was a significant ($p<0.05$) negative correlation between the treated time and survival of young snails hatched from eggs laid by snail treated to 40%, 80% of 24h LC_{50} of leaf powder, different organic extracts, and column purified fractions (Table-2).

Table 1: The effect of sub-lethal (40% and 80% of 24h LC_{50}) molluscicide of *S. surattens* leaf powder, organic extract (ether extract, chloroform extract, methanol extract, acetone extract, ethanol extract), and column purified fraction on the fecundity of the snail *L. acuminata*

Treatment (Sub-lethal concentration of 24h LC_{50} of 40 and 80%) [#]		Fecundity after 24h (eggs/20 snail)	Fecundity after 48h (eggs/20 snail)	Fecundity after 72h (eggs/20 snail)	Fecundity after 96h (eggs/20 snail)	Withdrawal after 96h treated snails Fecundity after 72h (eggs/20 snails)
Control		190.23±0.15	185.21±0.12	182.13±0.16*	184.65±0.11*	192.31±0.21
S. surattens (LP)	40%	+170.20±0.23*	168.22±0.15*	164.40±0.18*	160.21±0.19*	171.32±0.17
	80%	+140.22±0.18*	134.31±0.38*	133.46±0.19*	130.27±0.45*	146.38±0.19
Ether extract	40%	+155.38±0.56*	151.16±0.80*	148.60±0.37*	145.36±0.23*	160.16±0.40
	80%	+128.52±0.14*	127.88±0.34*	124.26±0.33*	122.27±0.20*	134.40±0.19
Chloroform extract	40%	+154.86±0.64*	150.64±0.43*	146.51±0.47*	143.20±0.22*	157.30±0.35
	80%	+130.51±0.88*	129.71±0.15*	127.19±0.84*	125.30±0.16*	135.45±0.26
Methanol extract	40%	+152.27±0.33*	150.60±0.34*	147.51±0.64*	144.92±0.52*	158.54±0.32
	80%	+126.60±0.22*	123.21±0.18*	120.92±0.52*	120.03±0.20*	132.43±0.28
Acetone extract	40%	+150.37±0.20*	147.52±0.72*	145.33±0.27*	144.65±0.34*	156.19±0.50
	80%	+127.20±0.28*	126.70±0.29*	124.55±0.40*	122.11±0.34*	134.26±0.40
Ethanol extract	40%	+110.40±0.22*	102.32±0.11*	95.20±0.32*	90.53±0.38*	122.85±0.80
	80%	+56.88±0.27*	53.50±0.29*	50.36±0.88*	48.40±0.35*	65.28±0.19
Column purified	40%	+60.51±0.39*	30.32±0.15*	-	-	71.60±0.22
	80%	+32.53±0.80*	15.22±0.20*	-	-	38.76±0.28

Each value is mean ± SE of six replicates. Each replicates represent the egg laid by the group of 20 snails. (*) significant ($P<0.05$) when the student "t" test was applied to treated and control groups. (+) product-moment correlation coefficient showed that there was a significant ($P<0.05$) negative correlation between the exposure period and fecundity of snail *L. acuminata*. (-) No fecundity was observed. [#] Kumar, 2021c. Abbreviations: LP- Leaf powder.

Table 2: The effect of sub-lethal (40% and 80% of 24h LC_{50}) molluscicide of *S. surattens* leaf powder, organic extract (ether extract, chloroform extract, methanol extract, acetone extract, ethanol extract), and column purified fractions on the hatchability and survival of the snail *L. acuminata* eggs obtained after 24h

Treatment (Sub-lethal concentration of 24 h LC_{50} of 40 and 80%)		Hatchability percentage (hatching period)	Percent survival after 24h	Percent survival after 48h	Percent survival after 72h
Control		100 (8-12)	100	100	100
S. surattens (LP)	40%	130.16±0.30(10-15)	125.11±0.28*	122.31±0.18*	120.540±0.32*
	80%	120.22±0.50(11-16)	115.28±0.16*	114.40±0.25*	112.77±0.24*
Ether extract	40%	125.22±0.45(10-15)	118.13±0.22*	116.19±0.45*	114.24±0.54*
	80%	119.82±0.50(11-16)	114.12±0.46*	111.21±0.42*	110.61±0.64*
Chloroform extract	40%	123.65±0.28(10-15)	116.71±0.43*	113.47±0.21*	112.84±0.54*
	80%	118.43±0.83(11-16)	114.54±0.35*	112.42±0.52*	111.65±0.32*
Methanol extract	40%	122.66±0.52(10-15)	117.30±0.21*	115.60±0.61*	112.60±0.29*
	80%	112.52±0.31(11-16)	114.10±0.19*	112.35±0.88*	111.04±0.25*
Acetone extract	40%	121.37±0.42(10-15)	118.61±0.42*	116.51±0.11*	112.80±0.16*
	80%	111.34±0.12(11-16)	114.50±0.27*	113.60±0.81*	111.30±0.90*
Ethanol extract	40%	115.33±0.70(10-15)	111.31±0.81*	102.51±0.66*	96.37±0.12*
	80%	104.63±0.65(11-16)	83.60±0.24*	60.45±0.25*	-
Column purified	40%	85.31±0.72(11-16)	-	-	-
	80%	56.93±0.25(11-17)	-	-	-

Each value is mean ± SE of six replicates. Each replicates represent the egg laid by the group of 20 snails (*) significant ($P<0.05$) when the student "t" test was applied to treated and control groups. (-) No fecundity was observed.
Abbreviations: LP- Leaf powder.

DISCUSSION

The results section demonstrates that the dried leaf powder of *Solanum surattense* and their organic extract (ether, chloroform, methanol, acetone, and ethanol), and column purified significantly reduced the fecundity of snail *Lymnaea acuminata*. The 40% of 24h LC_{50} of column purified anti-reproductive/molluscicidal components reduced the fecundity of snail *L. acuminata* within 72h. Kumar, (2021c) has been reported that dried leaf powder, organic extract (ether, chloroform, methanol, acetone, and ethanol), and column purified fractions of *S. surattense* are potent molluscicides against snail *L. acuminata.* Dixit and Gupta, (1982) have been reported that the anti-spermatogenic property of solasodine, an alkaloid which is found in the fruit of *S. surattense*. It also examines the administration of solasodine at 20 mg/kg to experimental animal models for 30 days resulted that testicular lesions and severe impairment of spermatogenesis. The biochemical analysis like glycogen, total protein, and sialic acid contents of the testis and epididymis were significantly reduced, whereas the level of testicular cholesterol was increased. The ethanolic extract of *S. surattense* was tested against *Plasmodium* in infected mice which have a significant reduction (Garedaghi and Khaki, 2014).

The leaf extract of *S. surattense* has larvicidal efficacy against *Culex quinquefasciatus* (Mahesh *et al.,* 2012). It's also having many alkaloids (Siddiqui and Faizi, 1983), sterols (Kusano *et al.,* 1973), saponines (Tupkari *et al.,* 1972), flavonoids and their glycosides (Debey and Gupta, 1936), tannins, gums (Sheeba, 2010). Although, the different preparation of *S. surattense* on invertebrate reproduction is not reported, yet it is clear from the present study that the phytochemicals of *S. surattense* enter the snail body through diffusion, and it ultimately affects the caudodarsal cells reducing the

release of the ovulation hormone, which may result in a decrease in the fecundity of the treated snails. The caudodarsal cell is responsible for the fecundity of host snail *L. acuminata* (Roubos *et al.,* 1981; Singh *et al.,* 2004; Kumar *et al.,* 2014). Several tannins bearing different families of plants have molluscicidal properties (Ayoub and Yankov, 1986). Bahuguna *et al.,* (2008) have been described that different leaf extract of *S. surattense* like petroleum ether, aqueous alcohol, and chloroform for antiulcer activity areas like pH, total acidity, free acidity, and ulcer. Suhas *et al.,* (2009) have been investigated that the methanolic extract of *S. surattense* shows antibacterial activity against gram-positive bacteria *Streptococcus aureus* and *Bacillus subtilis* at 50, 75, and 100 µg/ml concentrations. This plant is also used in the treatment of insomnia, cold, worms (Mathur and Agrawal, 2011), laxative, enlargement of liver, aphrodisiac activities (Kiritikar and Basu, 2005; Gupta *et al.,* 2011), anti-nociceptive, molluscicidal, and anti-fungal activity (Bhutani *et al.,* 2010).

The reduction of snail *L. acuminata* exposed to the sub-lethal treatment of leaf powder, different organic extracts, and column purified of *S. surattense* is due to its interference with the embryonic growth and development of the snails. The treated snails, young larvae were weak, unable to break the egg membrane/capsule, and died owing to starvation. Whereas, the young snails hatched from the treated egg masses showed a delay in attaining maturity in comparison with the control groups. Therefore, the low reproduction capacity in the exposed snails suggests that the phytochemicals/molluscicides of the *S. surattense* were able to control the population of host snail *L. acuminata* by inhibiting growth and development. The mother snails were transfer to freshwater for the next 72h after 96h exposure to the treatment which leads to a significant recovery in the fecundity. Therefore, the withdrawal experiments also indicate that the treatment of different preparations of *S. surattense* was reversible as the activity was restored within 7 days. Likewise, reversibility of the effects would be an added advantage in their use against aquatic target snails as they would cause only short-lived effects.

CONCLUSION

The present study demonstrates that the medicinal plant *S. surattense* has anti-reproductive properties against the freshwater host snail *L. acuminata*. The sub-lethal doses of leaf powder, organic extract (ether, chloroform, methanol, acetone, and ethanol), and column purified of *S. surattense* alter the reproductive capacity, inhibiting the development of snail eggs, growth of young larvae and these possess a capability of making them sterile in the host snail *L. acuminata*. It also causes embryo death during the developmental stage and inhibiting the hatching capacity in the snail.

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