

Noxiousness Valuation of Two Pharmaceutical Plants on White Garden Snail and Their Side Influences on Biochemical Parameters and Histopathological Changes in Brown Rat

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The current study aimed to evaluated the toxicity of *Abrus precatorius* (AP) and *Ricinus communis* (RC) plants on land snail (*Theba pisana*) and their effects on biochemical and histological changes in brown rat (*Rattus norvegicus*). Different concentration (1%, 1.5%, 2% and 2.5%) of AP and RC were used in this study to evluate their effects. The findings of this study pertained that the seeds of AP and RC had potential impact to control *T. pisana* and the LC_{50} of AP and RC had negative effects on many life cycle stages of *T. pisana*. The oviposition and new generation of *T. pisana* decreased by 81.2% and 62.5% and by 100% and 96.7%, while the infertility rate declined by 96.4% and 82.4% after treating *T. pisana* by AP and RC, respectively. Results also showed that AP and RC had weak influences on the liver, spleen, intestine, kidney and testis tissues of *R. norvegicus*. Furthermore, the effect was relatively higher for *A. precatorius* than *R. communis* on *R. norvegicus* males.

Keywords: Theba pisana, Rattus norvegicus, Abrus precatorius, Ricinus communis.

1. INTRODUCTION

Mollusks are well-known and widespread invertebrate animals in the world. Many species of them are very beneficial to the human race, whether aquatic or terrestrial. However, there are some types are harmful, especially terrestrial ones [1]. They can spread on many plants and cause mark damages [2], through feeding on them or transmitting diseases by the secreted mucous material. *Theba pisana* is one of the most harmful snails in Egypt and can widely distribute on fruit trees [3]. Several methods such as agricultural, mechanical and chemical have been applied to control the ground snails, and the use of poisonous baits is known as one of the effective methods.

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There are many herbal and medicinal plants are found in nature and some of them are used to control many invertebrate pests. Abrus preatorius and Ricinus communis are poisonous plants with marked toxicity against various pests and have given effective results in their control [4–9]. The seeds and foliage of A. precatorius are poisonous materials due to presence of toxic proteins such as abrin and ricin [10-12]. R. communis plant protects itself naturally against pests because its dry seeds are contained high concentrations of ricin protein, which consists of poison acid groups, including ricinoleic acid [13]. It is necessary to test the effect of A. precatorius and R. communis plants on mammals, which largely reflects their impacts on the human race, especially if they are integrated urgently in the control of pests. The study is going to provide some applied information about the use of pharmaceutical (medicinal) plants such as Abrus precatorius

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Mohamed et al.

and *Ricinus communis* to control the garden white snail and to evaluate their safe effects on mammals, which are presented in brown rat as an indicator animal. Besides it can investigate the toxic effects of *A. pecatorius* and *R. communis* seeds on the land snail (*T. pisana*) and also to evaluate their effects on some biochemical indications and histopathological fluctuations of *Rattus norvegicus* organs as pointers for toxicity.

2. MATERIALS AND METHODS

2.1. Plants Source and Preparation

The seeds of *Abrus precatorius* and *Ricinus communisn* were procured from druggist store and Moshtohour village, Qalyubia Governorate, Egypt, respectively. The seeds were carried out to remove impurities by cleaning and then dried seeds were carefully crushed by a mortar using pestle and prepared power using electric mixer. The obtained powder was stored in a Wise Mix Ball Mill device until further use.

2.2. Preparing of T. pisana Land Snail in the Lab

Individual adults of the ground snail *T. pisana* were obtained from different infested fruit trees and ornamental plants at Qalubia Governorate, Egypt. The snails were directly transferred to the laboratory in plastic cages contained a mixture of clay, sand and sawdust. After cleaning the individuals with running tap water, the best individuals with a similar size were selected and placed in cages and then fed with fresh lettuce leaves, which were changed daily for two weeks. The cages were covered with a perforated cloth to prevent the individuals from escaping. Before starting the experiment, snails were prevented from eating for seven consecutive days.

2.3. Effect of *A. precatorius* and *R. communis* on the Mortality of *T. pisana*

The tested seeds were presented to T. pisana in baits forms. 50 g of milk powder was mixed with four different concentrations (1, 1.5, 2 and 2.5%) of A. precatorius and R. communis powder in a replicate of six. 5 healthy adults with the same size and shape of T. pisana were placed on fresh and clean lettuce leaves contained 5 g of the previously prepared baits. In addition, six replicates for the control treatment were also made and the adults of T. pisana were fed on untreated lettuce leaves. All replicates were covered with tightly cloths to prevent the escape of individuals. The experiment was monitored on daily basis and the mortality rates were recorded post-treatment for one week. The killed snails were counted and eliminated. Mortality percentages were corrected as described by Abbott [14]. The achieved death numbers of T. pisana were exposed to probit analysis by a computer program to determine LC_{25} , LC50, LC90, LC95 values. The mortality was calculated according to the previous study of Mohamed et al. [15].

2.4. Effect of A. precatorius and R. communis LC₅₀ Values on Life Cycle of T. pisana

Adults of *T. pisana* snail were treated with LC_{50} of *A*. precatorius and R. communis to explain their effects on some aspects of life by preparing three groups of terrestrial snails, the first group was fed on lettuce leaves treated with LC_{50} from A. precatorius, the second group was fed on lettuce leaves treated with LC_{50} from *R. communis*, and the third group was nourished on un-treated lettuce leaves as a regulator. Four replicates were organized for each group, and inside each replicate a part of the wet mixture soil was placed and then follow up until mating token place between the two individuals. The parameters such as copulation percentage, egg masses percentage laid by T. pisana, average clutch depth in soil (cm), average number of eggs per clutch, incubation period (day), hatchability percentage, oviposition inhibition percentage, sterility percentage and the reduction percentage in new generation were recorded. Sterility percentage was calculated according to Chamber Lain's formula: as mentioned by Guirguis [16].

$$\%$$
Sterility = 100 - [a × b/A × B] × 100 (1)

Where a: No. of eggs/animal in treatment, b: % hatching/animal in treatment, A: No. of eggs/animal in control and B: % hatching/animal in control.

2.5. Preparing of Rattus norvegicus Rats

Adult males of *R. norvegicus* were collected from nearby villages, Qalubia Governorate and adapted to laboratory environments for 2 weeks before the experimental study.

2.6. Effect of LC₅₀ Concentrations of A. precatorius and R. communis on R. norvegicus

Males of *R. norvegicus* were randomly assigned to 3 groups with 3 replicates and each group contained 3 rats. The first and second groups were nurtured on a commercial feed mixed with LC_{50} concentrations of *A. precatorius* and *R. communis* but the third group was nourished only on the commercial feed. The commercial feed consisted i.e., protein (21%), fat (59%) and fiber (4.20%) etc. were fed to various groups of rats for 30 days.

2.7. Biochemical Parameters

All biochemical parameters were carried out in Dr. Mahmoud Abou El. makarem laboratory, Toukh, Qalubia Governorate, Egypt. Blood samples were drawn on serum tubes. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), urea and creatinine were spectrophotometrically determined as described by Madaki et al. [17].

2.8. Histological Studies

All histological studies were conducted in Attar Center for Medical Researches and Histopathology (ACMRH), Zagazig, Sharkia Governorate, Egypt. Samples from the vital organs (liver, spleen, kidney, intestine, and testes) of treated R. norvegicus males were collected 30 days after all treatments, including the control. The processing consisted of an initial 2 steps, fixation and dehydration. All samples except testes were secured in 10% neutral buffered formalin but testes were fixed in Bouin's liquidfor 48 h and preserver was detached by purified water for 30 min. By passing the preserved specimens through a classified series of ethyl alcohol, the tissues were dehydrated (70%, 90%) and 100%). The tissues were first subjected to 70% alcohol for 120 minutes, followed by 90% alcohol for 90 min, and then two one-hour cycles of absolute alcohol. After dehydration, the samples were cleared in numerous changes of xylene. It involved immersing tissue for an hour in a mixture of 50% alcohol and 50% of xylene, followed by 1.5 h in pure xylene. After that, the samples were immersed in molten paraffin wax, imbedded and blacked out. Circulatory irregularities, inflammation, degenerations, apoptosis, necrosis, and any other pathological alterations were evaluated using paraffin sections (4–5 μ m) stained with hematoxylin and eosin [18]. The Leica microscope was used to capture and photograph any histological changes.

2.9. Statistical Analysis

Completely randomized design (CRD) was used to design and perform the experimental section in replicates of 6. The statistical analysis was carried out using one-way ANOVA using SPSS, ver. 22. Duncan multiple range test was appointed at $P \le 0.05$ of significance level.

3. RESULTS AND DISCUSSION

3.1. Toxic Effects of A. precatorius and R. communis on T. pisana

Table I summed up the results of lethal concentration $(LC_{25}, LC_{50}, LC_{90} \text{ and } LC_{95})$ of *A. precatorius* and *R. communis*. *A.* the results indicated that the Faculty of Agriculture, Benha University, Egypt *precatorius* was more toxic compared to *R. communis* on and *T. pisana*. The values of $LC_{25}, LC_{50}, LC_{90}$ and LC_{95} were 0.905%, 1.567%, 4.447% and 5.977% respectively for *A. precatorius* for the first plant, these value are significantly lower as compared to *R. communis* i.e., 1.034%, 2.234%, 9.658% and 14.627% after 5 days of treatments. These findings are consistent with the study conducted by Francis et al. [19], those

confirmed that the success and efficiency of *R. communis* extract in controlling the Golden apple snail (*Pomacea canaliculata*). Dahi et al. [20] also indicated that *A. precatorius* had marked effects in controlling many invertebrate pests such as mollusks and insects, with the different forms of *R. communis* extracts had a high efficiency in increasing the mortality of terrestrial snail (*Monacha obstructa*).

3.2. Effect of A. precatorius and R. communis (LC_{50} values) on Life Cycle of T. pisana

The results for the effect of LC₅₀ values of A. precatorius and R. communis on some life cycle aspects of T. pisana under laboratory conditions are depicted in Table II. The efficacy of A. precatorius and R. communis start to appear directly from the beginning of experiment, where only 25% and 50% of treated individuals succeeded in copulation, respectively in comparison to untreated individuals. Moreover, marked decreases (12.5% and 37.5%) were found in egg masses percentage laid by T. pisana as results of A. precatorius and R. communis applications, respectively. The results also confirmed that the individuals treated with LC₅₀ of A. precatorius and R. communis lost the ability to dig in the soil to lay eggs, while the average depth of the clutches placed in the soil was 4.35 cm by untreated individuals. On the other hand, a high significant difference $(p \le 0.05)$ appeared between the treated and untreated T. pisana in average egg numbers/clutch. The egg numbers were 11 and 22.5 eggs/clutch after the treating with A. precatorius and R. communis, these were significantly lower a compared to control (58.38 eggs/clutch). The incubation period increased up to 19 and 18.75 days in A. precatorius and R. communis respectively, compared to 12.25 days for the control. In addition, the percentage of egg hatchability reached 18.2% in the case of A. precatorius and 43.2% for R. communis, but reached 94.68% in the control. This could be used as conclusive evidence to confirm the effect of A. precatorius and R. communis on egg hatching process. The oviposition inhibition percentage was increased upto 81.2% and 62.5% for A. precatorius and R. communis, respectively. The sterility rates in T. pisana were recorded 96.4% and 82.4%, while the reduction percentages in its new generations reached 100% and 96.7% due to using A. precatorius and R. communis treatments, respectively. These results are in agreements with outcomes of Dahi et al. [20], which indicated that R. com*munis* extract had many strong effects on some biological

Table I. The toxic effects of A. precatorius and R. communis on T. pisana under laboratory conditions.

		Lethal concentrations % and their 95% confidence limits						
Tested plants	LC ₂₅	LC ₅₀	LC ₉₀	LC ₉₅	Slope			
A. precatorius	0.905	1.567	4.447	5.977	2.832			
R. communis	(0.053-1.234) 1.034 (0.656-1.628)	(1.131-2.172) 2.234 (1.418-3.519)	9.658 (6.131–15.215)	(4.313–6.283) 14.627 (9.285–23.043)	2.024			

Table II.	Effect of A.	precatorius and R.	communis (LC50	values) on some	life cycle aspects	s of T. pis	ana under laboratory conditions.
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Treatments	(%) Copulation	(%) Egg clutches	M. of clutch depth (CM)	M. of eggs/ clutch	M. of I period (day)	(%) Hatchability	(%) Oviposition inhibition	(%) Sterility	(%) New G inhibition
A. precatorius	25.00 ± 75.00^{b}	2.50 ± 12.50^{b}	0.00 ± 0.00^{b}	11.00 ± 70.00^{b}	19.00 ± 0.00^a	$18.20 \pm 0.00^{\circ}$	81.2	96.4	100
R. communis	50.00 ± 28.87^{ab}	37.50 ± 23.94^b	0.00 ± 0.00^{b}	22.50 ± 10.00^{b}	18.75 ± 0.75^a	43.20 ± 1.80^b	62.5	82.4	96.7
Control	100.00 ± 0.00^a	100.00 ± 0.00^a	4.35 ± 0.21^a	58.38 ± 3.83^{a}	12.25 ± 0.85^b	94.68 ± 0.94^a	0.00	0.00	0.00

Notes: Means with different letter(s) i.e., a, b, c ... in the same columns are significantly different.

aspects of cotton leaf worm *Spodoptera littoralis*. Amer et al. [21] was also confirmed that the application of LC_{25} and LC_{50} doses from *A. precatorius* had negative influences on the life cycle of the two-spotted spider mite female, *Tetranychus urticae* through decreasing the laid egg numbers.

3.3. Toxicity Assessment of *A. precatorius* and *R. communis* (LC₅₀ Values) on Liver and Kidney Functions of *R. norvegicus*

The effects of of *A. precatorius* and *R. communis* (LC_{50} values) on the liver and kidney functions through determination of some biochemical changes in *R. norvegicus* blood are shown in Table III. The enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase), and ALP (alkaline phosphatase) were used to assess liver function. There are no notable distinctions (P = 0.05) were shown in values of ALT and ALP due to using LC_{50} of *A. precatorius* and *R. communis*, but they caused significant changes ($P \le 0.05$) in AST enzyme. Its value was 180.50 U/L in the control treatment, and then notably increased to 205.50 U/L and 220.67 U/L when *R. norvegicus* was treated with LC_{50} of *R. communis* and *A. precatorius*, respectively.

The Kidney functions were evaluated by determining levels of urea and creatinine in the blood. Data revealed significant changes in urea levels in *R. norvegicus* blood from 41.70 mg/dl in the control to 46.60 mg/dl and 43.60 mg/dl in LC₅₀ of *R. communis* and *A. precatorius*, respectively. In contrast, the creatinine levels showed no significant variations in the blood of *R. norvegicus* after application of LC₅₀ concentrations of *A. precatorius* (0.34 mg/dl) and *R. communis* (0.31 mg/dl) as compared with the control (0.35 mg/dl) at $P \le 0.05$.

From assessment the previous results, it was clear that effect of *A. precatorius* and *R. communis* on *R. norvegicus* liver and kidney functions was non-significant (≥ 0.05), as

no noticeable changes appeared, except some little effects on AST liver enzyme. These results established that A. precatorius had a slight higher effect on liver and kidney functions than R. communis. Outcomes of Sandhyakumary et al. [22] confirmed that R. communis extract did not cause any hepatotoxicity on rats as the GOT and GPT levels in their bloods were not changed. Raju et al. [23] was also confirmed that the extract of R. communis roots did not cause any changes or toxic effects on levels of AST, ALT, and ALP liver enzymes as well as levels of urea and creatinine in the blood of Wistar albino rat under laboratory conditions, which might indicate the safe use of R. communis on mammals. Madaki et al. [17] indicated that the use of A. precatorius leaves extract was relatively safe and did not distress liver and kidney functions of rats. The low doses of A. precatorius seeds extract had no toxicity on liver and kidney functions in adults of Wistar albino rats without any death [24].

3.4. Histopathological Findings of R. norvegicus Males

Control Group: Tissues sections of normal *R. norvegicus* males in the control group (untreated with *A. precatorius* or *R. communis*) were shown in Figure 1. The results reavealed that the, liver sections showed normal hexagonal plates of hepatocytes. The liver acinus, which was the functional unit had oval shaped. A shared border between two adjacent lobules, as well as the portal canals, formed its short arm. The long arm was a fictitious line drawn between two core veins. The hepatocytes were large and polyhedral cells form about 75–80% of the total cells of the liver. They contains about 2 and 4 nuclei with spherical shape and presented in the center of the cells. The lifespan of a hepatocyte was about five months. The perisinusoidal space (space of Disse) was formed when the hepatocytes extend villi into the perisinusoidal vascular space,

Table III. Effect of A. precatorius and R. communis LC_{50} values on some biochemical changes of R. norvegicus.

Treatments		Liver enzymes	Kidney function		
	ALT (U/L)	AST (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
A. precatorius	20.67 ± 0.44^{a}	220.67 ± 1.45^{a}	85.70 ± 0.40^{a}	46.60 ± 0.21^{a}	0.34 ± 0.01^{a}
R. communis	20.20 ± 0.31^{a}	205.50 ± 2.75^{b}	85.90 ± 0.45^{a}	43.60 ± 0.35^{b}	0.31 ± 0.01^{a}
Control	20.10 ± 1.04^a	$180.50 \pm 5.11^{\circ}$	85.60 ± 0.70^{a}	$41.70 \pm 0.85^{\circ}$	0.35 ± 0.02^a

Notes: Means with different letter(s) i.e., a, b, c ... in the same columns are significantly different.



Fig. 1. Photomicrograph from liver (A, B), kidney (C, D), spleen (E, F), intestine (G, H) and testis (I, J) of control *R. norvegicus*, showing normal histomorphology of the corresponding organs. The hepatic cords (light blue arrow), portal area (deep blue arrows), renal tubules (deep blue arrows), glomeruli (light blue arrows), splenic white pulp (deep blue arrows), red pulp (light blue arrows), intestinal villi (deep blue arrows, intestinal mucosa (light blue arrows) and testicular seminiferous tubules (deep blue arrows) and spermatozoa (light blue arrows), all are seen with apparent normal arrangement and histological structures). H&E $\times 100$, 200, 400.

increasing the rate of fluid exchange. The biliary tributaries tree was a system consisting of channels that carries bile from the source, this being the hepatocytes, all the way to the gallbladder and intestines (Figs. 1(A, B)).

Cortical and medullary structures were visible in kidney slices. The glomerulus, which was open in the proximal and distal tubule, in the descending and ascending Henle loop, and in the straight parts, constituted the functional unit. The collecting channels, which were output in the papillary ducts, connected the nephrons. In the renal pelvis, the papillary ducts connect to the tip of the renal papilla. Transitional epithelium bordered the renal pelvis, which ran parallel to the ureter. The renal papilla in rats may be lengthy and protrude into the ureter's first section. Bowman's capsule surrounding the glomerulus tufts, which could show sexual dimorphism under the effect of testosterone (parietal epithelial layer generally found in males). Bowman's capsule parietal cells were found to be flattened in the vascular pole and cuboidal in the urine pole of the glomeruli (Figs. 1(C, D)).



Fig. 2. Photomicrograph from liver (A, B), kidney (C, D), spleen (E, F), intestine (G, H) and testis (I, J) of *A. precatorius* showing at LC_{50} hepatic portal round cells aggregations, mild biliary proliferation and hepatocellular hydropic degeneration (dark and light blue arrows). Renal tubular degeneration, hyaline cast formation and tubular dilatation are seen (dark and light blue arrows). The spleen shows moderate depletion of the white pulp lymphoid cells (dark blue arrows). The small intestine shows villitis, villous stratification and increase in number of goblet cells. Focal testicular edema, atrophy, beside degenerative and necrotic changes in spermatogonia and spermatocytes of some seminiferous tubules are seen (dark and light blue arrows). H&E $\times 100$, 200, 400.

Mohamed et al.

Spleen sections demonstrated a red pulp composed of a splenic cords and venous sinuses. The splenic cords were formed from reticulin fibers, reticular cells, and associated macrophages (monocytes). The reticular cells are myofibroblasts and might play a role in splenic contractility. It also comprised the most important immunologically functional white pulp which subdivided into the PALS, the follicles, and the marginal zone. It was composed of lymphoid cells, macrophages (monocytes), dendritic cells, plasma cells, arterioles, and capillaries in a reticular framework similar to that found in the red pulp (Figs. 1(E, F)).

The small and large intestines are separated by three layers that run the length of the intestine (mucosa with submucosa, muscular and serous). The mucosa had lining epithelium and fibrovascular stroma, which was divided from the submucosa by the "mucosal muscular lamina." Connective tissue involving blood arteries, lymphatic vessels, and nerves created the submucosa. The muscle was made up of the inner circular layer. The thin layer of peritoneum formed the serosa (Figs. 1(G, H)). Testes sections showed normal testicular structures with preserved seminephrous tubules which appeared lined by normal spermatogonia, spermatocytes, spermatids and sertoli cells; they contained variable number of mature spermatozoa in their lumina. ledying cells, intesrstitial tissue and vascular structures were normal (Figs. 1(I, J)).

3.5. Effect of *A. precatorius* LC₅₀ on *R. norvegicus* Histomorphologic Changes

The examined tissues sections of *R. norvegicus* males which treated with *A. precatorius* were shown in Figure 2. The sections indicated that they were some histomorphologic changes were occur by hepatic portal round cells aggregations, mild biliary proliferation and hepatocellular

hydropic degeneration. Renal tubular degeneration, hyaline cast formation and tubular dilatation were seen. The spleen showed moderate depletion of the white pulp lymphoid cells. The small intestine showed villitis, villous stratification and increase in number of goblet cells. Focal testicular edema, atrophy, beside degenerative and necrotic changes in spermatogonia and spermatocytes of some seminiferous tubules were seen. Although some changes occurred in the tissues of *R. norvegicus* in *A. precatorius* treatment compared to the control group, no deaths were reported among the treated individuals throughout the experimental period. This might make the application of A. precatorius could be safely for mammals at its little doses. These histological explanations are in line with previous findings of Shazia et al. [24]. They proved that the small doses of A. precatorius did not cause any effect on heart, liver, lungs, kidneys, spleen, stomach, intestines, and brain organs of wistar albino rats.

3.6. Effect of *R. communis* LC₅₀ on *R. norvegicus* Histomorphologic Changes

The changes in *R. norvegicus* tissues which treated with *R. communis* are showed in Figure 3. The sections revealed some minor histomorphologic variations compared to that of the control group. However, some sections showed congested hepatic blood vessels and hepatocellular cloudy swelling. The spleen revealed mild depletion of the white pulp lymphoid cells. The small intestine showed some stunt villi and increases in numbers of goblet cells. No abnormal changes were recorded in the kidneys and testes in *R. communis* treatment. No mortalities had appeared among the treated *R. norvegicus* males. Thus, histological results proved that *R. communis* is safe as it caused some minor and ineffective changes in the liver, spleen and small



Fig. 3. Photomicrograph from liver (A, B), kidney (C, D), spleen (E, F), intestine (G, H) and testis (I, J) of *R. communis*, showing at LC_{50} congested hepatic blood vessels and hepatocellular cloudy swelling (dark and light blue arrows). The spleen shows mild depletion of the white pulp lymphoid cells (dark blue arrows). The small intestine shows some stunt villi and increase in number of goblet cells (dark and light blue arrows). H&E ×100, 200, 400.

intestine, and also did not cause any abnormal changes in the kidneys and tests. The result of present investigation are in line with previous finding of Raju et al. [23] those specified that the use of R. *communis* extracts with low doses had no marked effects on many organs of Wistar albino rats.

4. CONCLUSION

The use of A. precatorius and R. communis seeds against the land snail, T. pisana had great effects on many aspects in the life cycle of T. pisana, when LC50 doses of these seeds were used. The significant decreases were recorded in copulation, eggs masses, eggs per mass, eggs depth in soil and hatchability of T. pisana. On the contrary, there were significant increases were notice in the incubation period, oviposition inhibition as well as new generation inhibition and sterility. In the case of R. norvegicus males, there were no significant differences observed in liver and kidney functions between A. precatorius and R. communis and control treatment. As for the anatomical study, some simple histological changes were appeared in some tissues of rats. All examinations confirmed that effects of A. precatorius were higher than those of R. communis on snails or rats. The results could indicate that A. precatorius and R. communis might be used safely to control the snail pests without harmful impacts on mammals at the used low levels in this study.

Ethical Compliance

The experimental study under the supervision of the ethics committee of animal experimental managements at Faculty of Agriculture, Benha University, Egypt.

Conflicts of Interest

There are no conflicts to declare.

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