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CLINICO-PATHOLOGICAL AND HISTOLOGICAL OBSERVATIONS IN *OREOCHROMIS NILOTICUS* EXPOSED TO THE MOLLUSCICIDAL ACTIVITY OF *ANAGALLIS ARVENSIS*

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SUMMARY

Exposure of the Nile tilapia (*Oreochromis niloticus* = *O. niloticus*) to dry powder suspension of *Anagallis arvensis* (*A. arvensis*) at concentrations of 50 ppm and 100 ppm for 24 and 48 hours was under consideration for studying the reaction of this fish against the used plant. *A. arvensis* was previously succeeded as an effective molluscicide for control of Schistosoma and Fasciala snails. The present study aimed to test the effect of the molluscicidal doses of Saboon El- Gheit (*A. arvensis*) on some serum parameters and some tissue histopathological observations of *O. niloticus* to clarify whether this plant is safe, non toxic and fit for this type of fish or not. *O. niloticus* which were brought from Bahr Muess at Zagazig City to the working place where, they were acclimatized and exposed to the plant suspension showed no clinical symptoms and no patho-anatomic signs externally or internally after exposure periods and at sampling. However, the estimated levels of serum parameters in the studied fish: glucose, total lipid, total protein, total albumin, globulin, albumin / globulin ratio (A/G), uric acid, urea and creatinine showed no significant changes after 24 and 48 hours of exposure in comparing with controls. Whereas, serum enzyme activities of aminotransferases (aspartate aminotransferase = AST and alanine aminotransferase = ALT) and alkaline phosphatase (AP) in addition to values of serum electrolytes (Ca^{++}, P^{++} Mg^{++}, Na^{+}, K^{+}, and K^{+}/Na^{+} ratio) presented insignificant deviations at the same periods of sampling. Although, there are some slight deviations in some above measured parameters, but they are non significant.

The histopathological examination of gills, liver, spleen and skeletal muscles of *O. niloticus* in the present research exhibited non-significant lesions at the time of sampling. Therefore, all above re-

sults revealed that the dry powder suspension of *A. arvensis* at molluscicidal concentrations has neither toxic nor hazard effects for *O. niloticus* allover the conditions of this study.

INTRODUCTION

Schistosomiasis has become the most important and prevalent parasitic disease in the world (Brown, 1971) as it causes a great loss in economy and manpower where it prevails (WHO, 1986). A variety of methods for control of this disease have been proposed including chemotherapy, immunization and snail control. The snail intermediate host of Bilharziasis represents the weakest link in the parasite life cycle and its breakage seems to be the most effective mean available for reducing transmission of the disease. Hence, the restriction of the infection according to the report of WHO (1967) and the complete eradication of the snails (Bilharziasis intermediate host) could be achieved through the following means: -

1 - Biological agents such as competitors, predators, parasites or diseases (El-Dafrawy, 1989 and Abd El-Hamid, 1989).
2- Environmental agents by which the snail habitat is rendering unsuitable for their maintenance (Nojima and Sato, 1981).
3- Chemical, organic and synthetic compounds, which have been currently used as molluscicides such as Copper sulphate, Sodium pentachlorophenate, Frescon and Bayluscide, which have some disadvantages represented by their high cost, environmental pollution and their higher fish toxicity (Ghazaly and Said, 1995 and Abd El- Aziz et al., 1997). Also, the possible development of resistance of the snails against different molluscicides (Kloos and Mc Cullough, 1982) could be awaited. Therefore, and from the economical and ecological points of view these chemicals were almost replaced by plant molluscicides (Mc Cullough and Mott, 1983).

Chinese farmers currently use few plant molluscicides regularly for snail control as part of a national programme (Cheng,1971) without studying their effects upon the non-target organisms especially fish. The need for new and different plant molluscicides will meet the recently defined prerequisites of the ideal mollusicides (WHO, 1983; Hostettmann, 1984 and Marston and Hostettmann, 1985). Forty wild Egyptian plants from seventeen families were preliminary screened for the molluscicidal activity against snails (Tackholm, 1974). Three plants only are known to have high active molluscicidal potency than the other plants and *Anagallis arvensis latifolia* (which is used in the present study) is the most active one (Sedki, 1994). It is very common in the Nile Valley including the Delta, Upper Egypt and Fayoum Province and it is familiar to the rural populatior (Halim et al., 1984). *A. Arvensis* is used in the folk medicine for many purposes as diuretic, diaphoretic and expectorant (Launert, 1981); fo

treatment of severe diarrhea (Abu- Zeed, 2000); for liver complaints (Yamada et al., 1978); antifungal (Staron et al., 1969); antiviral properties (Amoros et al., 1987) and tumor narcotizing capacity (Grieve, 1976). Different parts of *A. arvensis* showed that the leaves dry powder is the most active against snails and the roots showed the lowest activity. The use of the whole parts of the mature plant in the preparation of the dry powder suspension at concentration of 100 ppm for 24 hours has feasible, economic and productive molluscicidal activity with relatively stable potency under simulated field conditions (Sedki, 1994). This molluscicidal activity could be attributed to the presence of one or more of active substances, which were separated by El- Sayed (1989) as triterpenoid saponins, flavonoides and glycosides. Shoeb et al. (1986) recorded the first economic value of the aqueous suspension of *A. arvensis* dry powder against *Biomphalaria alexandrina*, while, Shoeb et al. (1989) preliminary carried out its field application in Sharakia Province at concentration of100 ppm to obtain100% kill of snails as proved also by Sedki (1994) without need to pass through the complicated processes of extraction. The plant molluscicides are characterized by their highly effectiveness, rapidly biodegradation (Sedki, 1994) and less expensive than chemical, organic and synthetic ones (Mc Cullough et al., 1980) especially in developing countries. However, it is preferable to apply *A. arvensis* as plant molluscicide during spring and summer months, where the water temperature is relatively high to produce optimum results in snail control with minimal concentration of the plant dry powder suspension (Abd El- Raheem et al., 1979 and Nojima and Sato, 1981). Therefore, the aim of the present work is to study the effect of exposure to *A. arvensis* on some serum physiological and biochemical parameters as well as the histopathological reactions of the Nile Tilapia (*O. niloticus*).

MATERIAL AND METHODS

A) Plant material:

Anagallis arvensis latifolia, Family: *Primulaceae*, which is represented by only four genera, where *Anagallis* is one of them (Tackholm, 1974). Its name is Scarlet pimpernel in English and Saboon El- Gheit in Arabic and it is widely distributed in winter crops and characterized by its blue flowers (Abu- Zeed, 2000).

The plant, used was collected during January 2002 from privet clover and bean fields in Kaniate Village, El - Sharkia Province, where, *A. arvensis* was chosen to prepare a dry powder suspension from the whole parts of the plant. According to the method of Sedki (1994), collected plants were left to dry at room temperature in shad, then, they were very finely powdered by electric blender, sieved several times and the end result powder was kept in glass container. As well as the report of Shoeb et al. (1986), the dry powder

stock suspension (solution) of 1000 ppm (gm / lit of water) was freshly prepared on the base of weight / volume in dechlorinated tap water of PH 7.5 - 7.7. Where, concentrations expressed in term of part per million (ppm) that would permit the computation of used concentration values were prepared(WHO.1965).

B) Fish:

A total number of 36 adult apparently healthy Nile tilapia (*O. niloticus*) of both sexes with average body weight of 100-150 g were used in this study. They were brought from Bahr Muess, the branch of the Nile River at Zagazig city, Egypt. The fish were transported alive to Animal Health Research Institute in Zagazig in aerated plastic tanks under suitable transportation conditions to minimize the stress effect. Fish were divided into 3 equal groups and each group was subdivided into two equal subgroups. Each subgroup of 6 fish in number was kept in plastic tank of 250 liters capacity, as well as fish were acclimatized for 7 days for laboratory conditions before beginning the study. According to Stoskopf (1993), at the beginning of the acclimatization period fish were subjected to copper sulphate solution bath as 3 mg/ L for one hour to stop fungal and parasitic activities. Also, fish were subjected to a prophylactic dose of chloramphenicol as I gm /10 L of water for 48 hours to prevent bacterial infections especially after transportation.

Each tank containing fish was supplied with dechlorinated tap water and an air pump for aeration allover the periods of acclimatization and exposure to the suspension. Fish were kept at feeding commercial ration in the form of artificial pellets at ratio of 2% of fish body weight allover the period of study except 24 hours before collection of samples, feeding was stopped. The first group of fish was kept as control; the second and the third groups were exposed to freshly prepared dry powder of *A. arvensis* plant at concentrations of 50 ppm and 100 ppm for 24 and 48 hours for each group. Six fish were collected from each subgroup at 24 and 48 hours post exposure, then, blood samples were collected from caudal vein of fish and serum was separated by centrifugation of the blood samples at 3000 rpm for 15 minutes and kept in vials under deep freezing at -20*c until analysis. Tissue specimens of gills, liver, spleen and muscles were rapidly removed from each fish of control and exposed groups and fixed in10%neutral buffered formalin for 24- 48 hours. Gills were decalcified in formic acid solution (Roberts, 1978). Paraffin sections of 3- 5 um thick of collected fixed specimens organs were prepared and stained with hematoxylin and eosin and examined microscopically.

C) Biochemical analysis:

Using kits, serum levels of glucose, total lipids, total protein, total albumin, uric acid, urea

and creatinin were estimated according to Trinder (1969), Schmit (1964), Doumas et al. (1981), Ratliff and Morris (1973), Barham and Trinder (1972), Fawcett and Scott (1960) and Weissman and Pilleggi (1974) respectively. AST and ALT activities were determined calorimetrically according to Schmidt and Schmidt (1963). AP activity was measured according to Marsh et al. (1959). Electrolytes (Na^+, K^+, Ca^{++} and Mg^{++}) were measured using atomic absorption (Perkin Elmer, 2280) according to Fernandez and Kahn (1971), while, P^{++} is estimated according to Tiez (1970). Globulin concentration was calculated from the total protein level minus the total albumin concentration.

D) Statistical analysis:

Student's t-test according to Scendcor and Cochran (1967) was used to analyze the given data of this study.

RESULTS AND DISCUSSION

Exposure of *O. niloticus* for *A. arvensis* dry powder at concentration of 50 ppm and 100 ppm for 24 and 48 hours did not show any obvious clinical symptoms and patho-anatomic appearance externally or internally in exposed fish. Also, some serum analytical biochemical and physiological parameters and some tissue histopathological observations are reported in this study compared with controls in the following: -

A) Concerning the serum biochemical parameters in exposed *O. niloticus,* table (1) showed that there were no significant deviations in serum levels of : glucose, total lipid, total protein, total albumin, globulin, uric acid, urea, creatinin and albumin/ globulin ratio (A/G) on response to the exposure for *A. arvensis* dry powder suspension and 100 ppm for 24 and 48 hours.

B) The serum electrolytes (Ca^{++}, P^{++}, Mg^{++}, Na^+, K^+ and K^+/ Na^+ ratio) were not significantly changed in exposed *O. niloticus* as shown in table (2) in response to exposure for *A. arvensis* as molluscicide for 24 and 48 hours at concentrations of 50 ppm and100 ppm.

C) Liver enzyme activities of aminotransferases (AST and ALT) and AP in the serum of *O. niloticus* had no significant alterations after exposure to the molluscicide concentrations of A. arvensis for 24 and 48 hours as represented in table (3) as well as serum biochemical parameters and electrolytes.

D) Exposure of *O. niloticus* to the molluscicidal concentrations of *A . arvensis* at 50 ppm and 100 ppm for 25 and 48 hours did not show significant pathological lesions in different examined tissues which were represented as following: -

1) Gills at concentrations of 50 ppm as shown in Fig (1) and 100 ppm for 24 hours

showed normal architecture, while, the exposure to the same concentrations for 48 hours exhibited non-significant slight and non-siginficant some hemorrhage as indicated in Fig (2) and Fig (3) respectively.

2) Liver as shown in Fig (4) and Fig (5) at concentration of 50 ppm for 24 and 48 hours revealed non significant slight congestion and non significant few mononuclear cell infiltration respectively, while, at concentration of 100 ppm for 24 and 48 hours exhibited no pathological changes.

3) Spleen at concentrations of 50 ppm for 24 and 48 hours and 100 ppm for 24 hours showed normal architecture as revealed in Fig (6), but, after 48 hours of exposure to concentration of 100 ppm spleen exhibited non significant few mononuclear cell infiltration as shown in Fig (7).

4) Muscles at concentrations of 50 ppm and 100 ppm for 24 and 48 hours showed no any pathological changes revealed as normal architecture shown in Fig (8).

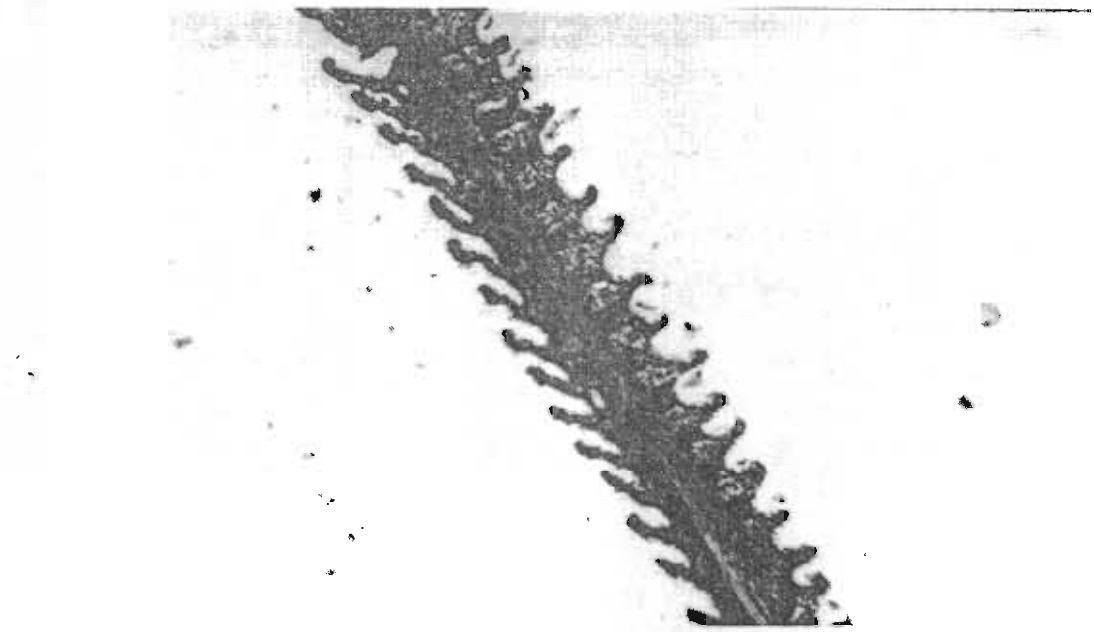

Fig (1): Gill of *O. niloticus* after exposure to *A. arvensis* at concentration of 50 ppm for 24 hours showed normal architecture (x 100).

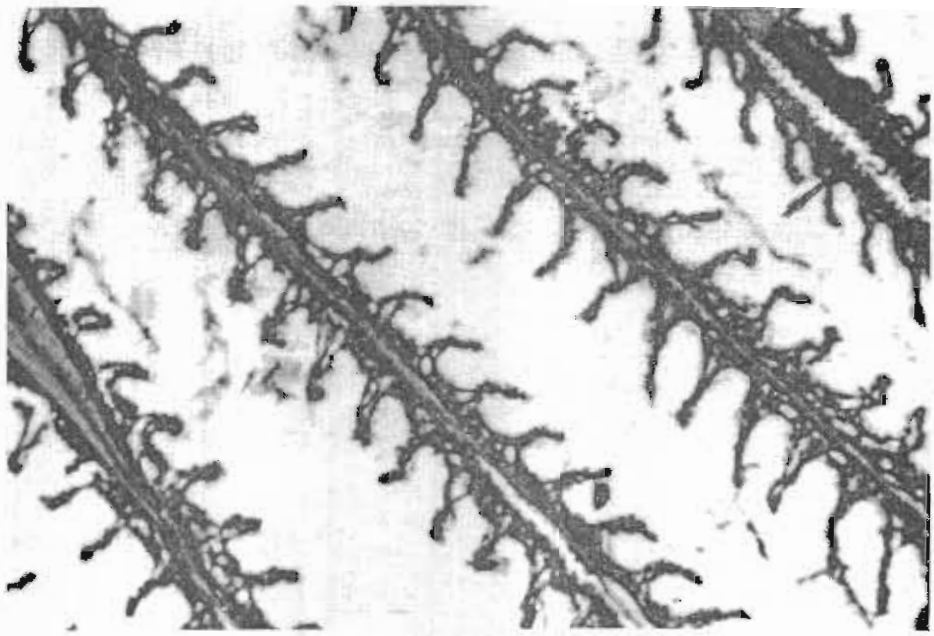

Fig (2): Gill of *O. niloticus* after exposure to *A. arvensis* at concentration of 50 ppm for 48 hours showed slight haemorrhage (x 200).

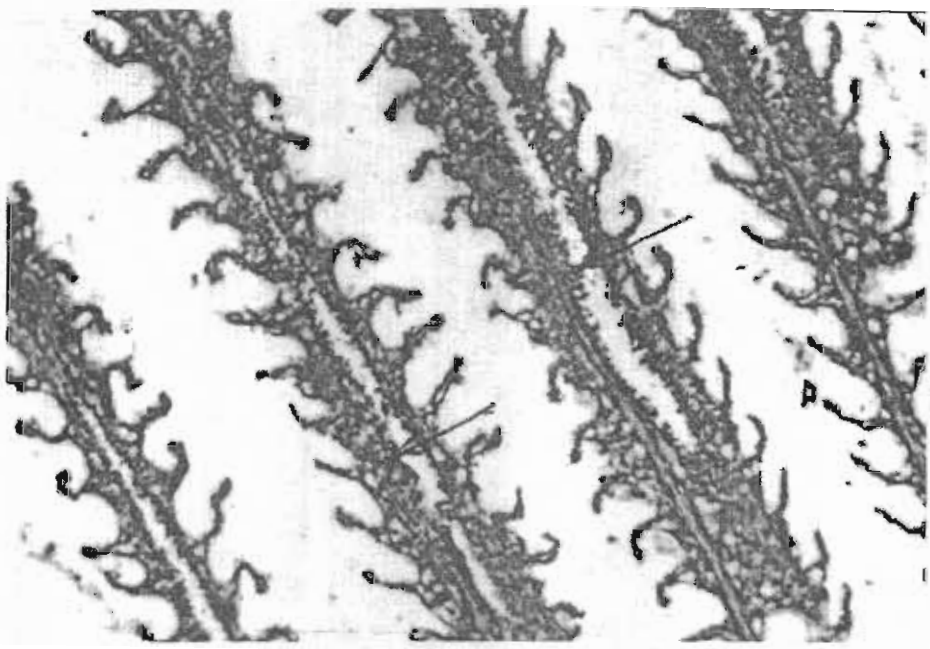

Fig (3): Gill of *O. niloticus* after exposure to *A. arvensis* at concentration of 100 ppm for 48 hours showed some haemorrhage (x 200).

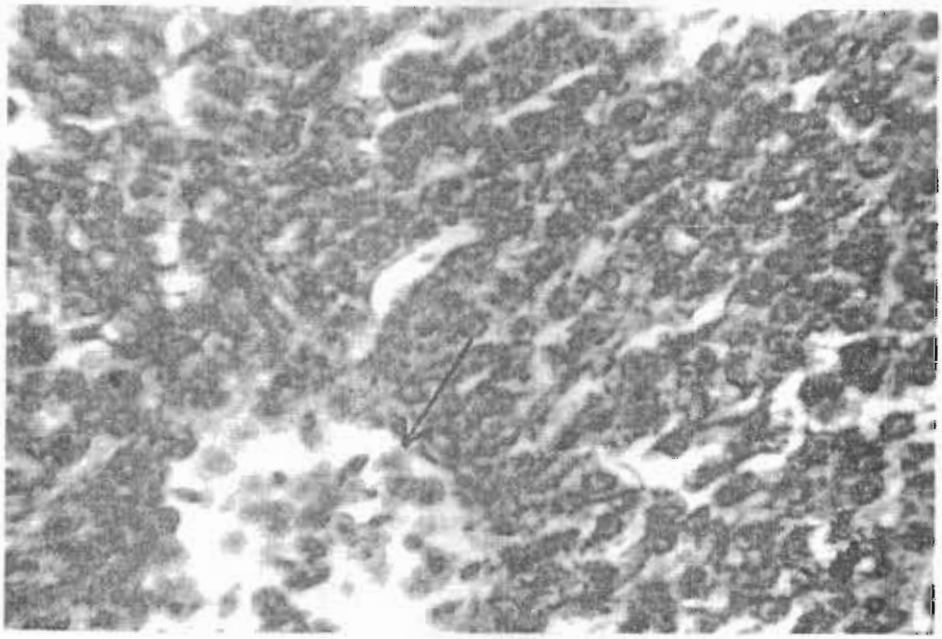

Fig (4): Liver of *O. niloticus* after exposure to *A. arvensis* at concentration of 50 ppm for 24 hours showed slight congestion (x 400).

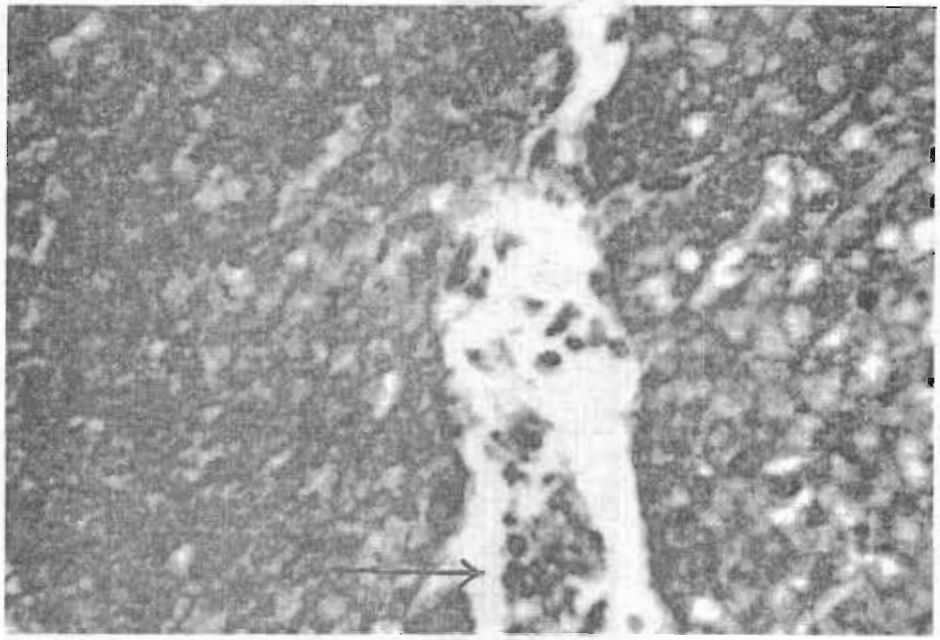

Fig (5): Liver of *O. niloticus* after exposure to *A. arvensis* at concentration of 50 ppm for 48 hours showed few mononuclear cell infiltration (x 400).

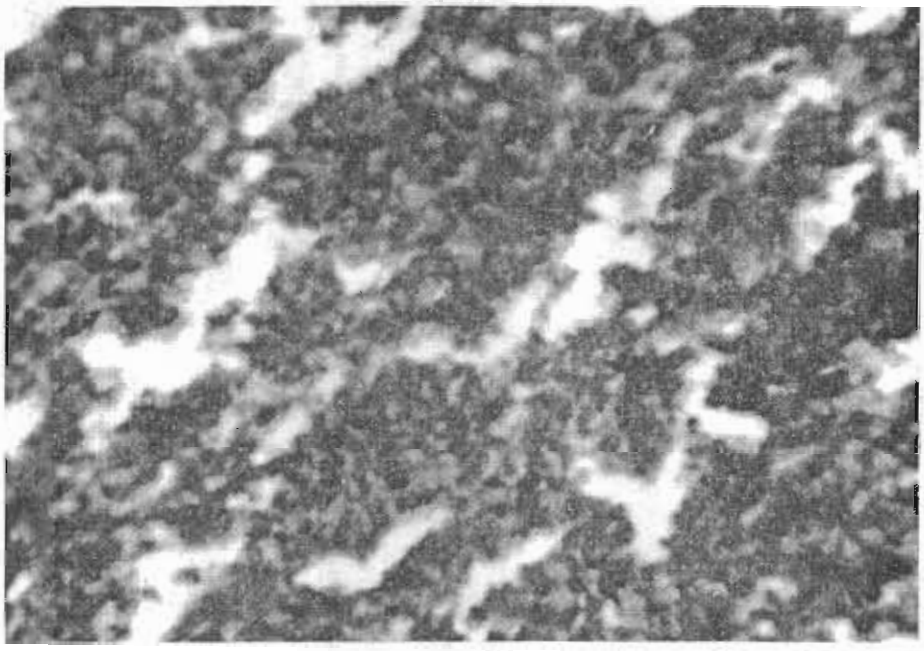

Fig (6): Spleen of *O. niloticus* after exposure to *A. arvensis* at concentration of 50 ppm for 48 hours showed normal architecture (x 400).

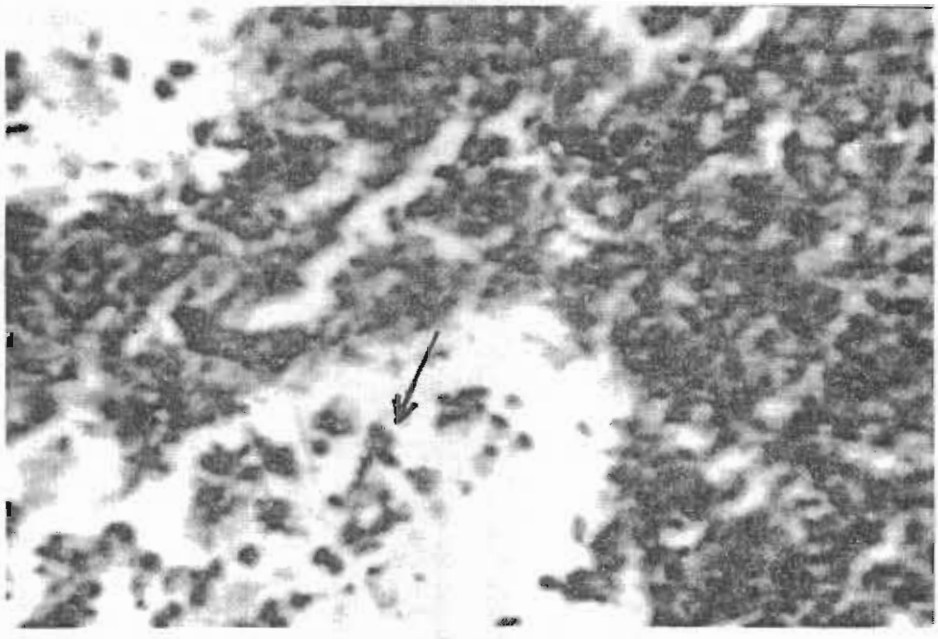

Fig (7): Spleen of *O. niloticus* after exposure to *A. arvensis* at concentration of 100 ppm for 48 hours showed few mononuclear cell infiltration (x 400).

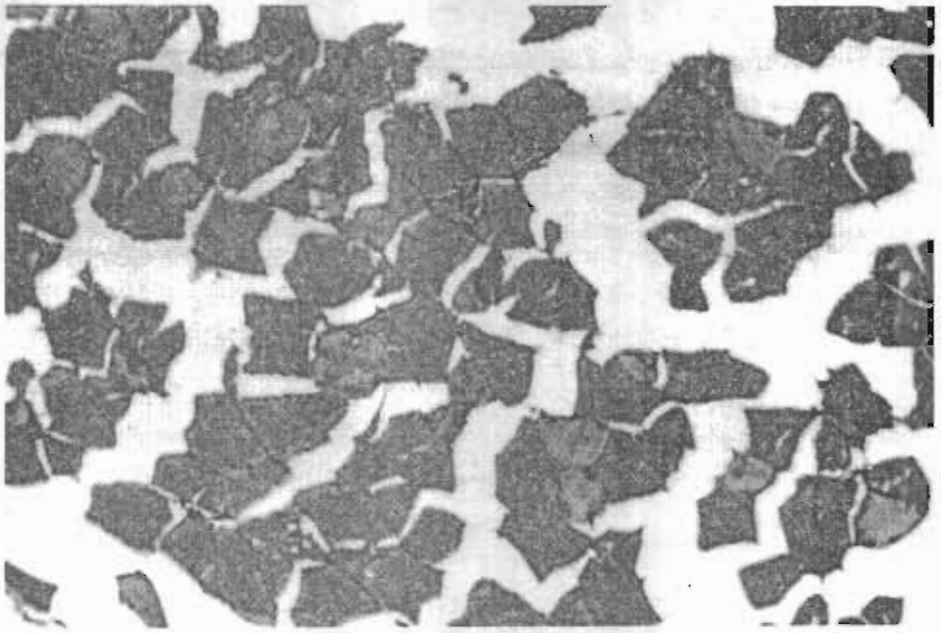

Fig (8): Muscles in cross section of*O. niloticus* after exposure to *A. arvensis* at concentration of 100 ppm for 48 hours showed normal architecture (x 100).

Thus these all above obtained histopathological observations exhibited no significant lesions in examined tissues of *O. niloticus* in response to exposure to *A. arvensis* as a molluscicide.

As the literature concerning the effect of exposure of the *O. niloticus* fish to *A. arvensis* as a molluscicidal plant is almost lacked, the present study could be regarded as the first study on the effect of this plant on fish under Egyptian conditions.

In fish blood glucose level is considered as a best and typical sensitive reliable indicator of environmental stress (Hattingh, 1976) and level of lipids are also important to reflect the physiological fish capacity (Schreck and Mayle, 1990). Total protein, total albumin and globulin are taken in consideration as indicators of hepatic condition and they are involved in the architecture, physiology, metabolism and immunity of the cell (Mommsen and Walsh, 1992), where, their synthesis and secretion are attributed to liver efficiency and any deviations in their serum levels indicating to affections, damage and \ or dysfunction of liver (Alvan, 1986). Because of a large proportion of serum enzymes including AST, ALT and AP are derived from the liver and the measurement of their some activities have been proved to be an useful estimate in diagnosis of liver diseases, affections, damage or dysfunction and also its efficiency (Koizumi et al., 1994) in addition to the physiological status of kidney (Sandnes et al., 1988). Serum electrolytes (including Ca^{++} , P^{++},

Mg^{++}, Na^{++} and K^{++}) play an important role in ionic balance of the cell and stress changes in osmoregulation of gills in fish, where, these changes can be manifested by altered plasma ion concentrations (Heath, 1987 & Abu El- Ella, 1996) and consequently, ionic equilibrium in the serum of fish means no gill impairment and not stressed leading to no any disturbances in their ion regulation.

Some studies were carried out on the effects and the toxicity of some other plant molluscicides on some species of fish. Daffala and Amin (1976) reported that seeds of *Croton macrostachys* have toxicity to human but it is nontoxic to *O. niloticus* at the molluscicidal concentrations. Shalabi et al. (2000) recorded that the Tilapia nilotica (*O.niloticus*) didn't suffer from any clinical symptoms, patho-anatomic signs and no biochemical responses of toxicity on exposure to Ammi majus fruit aqueous extract at its molluscicidal doses. Also, the molluscicidal activity of the different extracts of *Gardenia lutea* fruit (*Rubiaceae*) against *B. truncatès* and *B. pfeifferi* snails have no toxicity to fish at the snail lethal dose and no apparent clinical or histopathological signs of toxicity were observed when experiment rabbits were fed on its fruit pulp (Ahmed et al., 1984). On the other hand, *Entada phaseoloides* is lethal against snails in Philippines at low concentrations, but it kills exposed fish species below its molluscicidal levels (Yasuraoka et al., 1977). In addition, Jurberg et al. (1986) reported that the aqueous solution of the latex of *Euphorbia tirucalli* showed molluscicidal activity on *B. glabrata* at LD90 of concentration 85 ppm and has toxicity for fish similar to that of Bayluscide and copper sulphate. While, the seed of *Croton tiglium* (the potent plant molluscicide) are highly toxic to fish and mammals and are carcinogenic (Yasuraoka et al., 1980). The molluscicidal levels of the leaves, berries and bark from the tree *Apodytes dimidiate* as reported by Pretorius et al.(1991) caused mortality at LC10 of fish Oreochromis mossambicus after one hour of exposure.

As shown in this research, the obtained data and histopathological observations reflect no significant reactions of *O. niloticus* to the presence of the tested suspension of *A. arvensis* and this means that the molluscicidal doses of this plant may not be stress, toxic, anxious or haven't potential action in the ambient ecosystem of the Nile tilapia. The absence of any adverse histopathological observations in the vital organs of *O. niloticus* are in harmony and logically support the biochemical and physiological analysis of the serum and both with clinical and anatomical examinations and this can be attributed to the safety, non toxicity and fitness of *A. arvensis* at molluscicidal doses for *O. niloticus*. Then the end results of the present study is In harmony and agreement with the conclusion of Sedki (1994) that *A. arvensis* has no effective mammalian toxicity, where, there was no mortality, no clinical

Table (1): Effect of the exposure to the molluscicidal concentrations of *A. arvensis* of some serum biochemical parameters of *O.niloticus*.

Groups	Controls		Exposure to 50 ppm		Exposure to 100 ppm	
Subgroup / Parameters	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Glucose mg/dl	117.363+/-16.345	124.025+/-16.154	114.695+/-12.102	130.358+/-15.422	123.363+/-12.210	131.558+/-17.360
Total lipids g/dl	4.863+/-0.468	4.604+/-0.612	4.678+/-0.538	4.492+/-0.598	4.788+-=/-0.541	4.380+/-0.584
Total protein g/dl	3.997+/0.462	3.928+/-0.474	4.397+/-0.344	4.095+/-0.430	4.547+/-0.817	3.815+/-0.503
Total albumin g/dl	1.309+/0.045	1.354+/-0.046	1.341+/-0.046	1.264+/-0.044	1.277+/-0.044	1.399+/-0.047
Globulin g/dl	2.688+/0.417	2.574+/-0.428	3.056+/-0.298	2.831+/-0.386	3.270+/-0.773	2.416+/-0.456
A/G Ratio	0.486	0.526	0.438	0.446	0.390	0.579
Uric acid mg/dl	2.101+/-0.847	2.203+/-0.230	2.181+/-0.440	2.353+/-0.447	2.171+/-0.279	2.253+/-0.379
Urea mg/dl	12.505+/-3.633	15.067+/-3.284	13.005+/-4.589	15.367+/-5.557	13.305+/-3.887	16.700+/-4.756
Creatinin mg/dl	0.490+/-0.043	0.519+/-0.136	0.502+/-0.044	0.531+/-0.139	0.478+/-0.042	0.507+/0.133
Each value represents the mean +/-SE (Standard error of the mean).						
The mean value represents 6 fishes in number for each subgroup.						

Table (2): Effect of the exposure to the molluscicidal concentrations of *A. arvensis* on some serum electrolytes of *O.niloticus*.

Groups		Controls		Exposure to 50 ppm		Exposure to 100 ppm	
Parameters \ Subgroup		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
Ca^{++}	mg/dl	16.000+/-1.767	15.088+/-1.576	15.034+/-1.052	17.015+/-1.042	16.367+/-1.810	17.373+/-1.811
P^{++}	mg/dl	15.733+/-1.670	13.974+/-1.678	16.451+/-1.670	16.802+/-2.512	15.047+/-2.634	16.185+/-2.920
Mg^{++}	mEq/L	2.179+/-0.108	2.132+/-0.114	2.126+/-0.106	2.028+/-0.114	2.072+/-0.103	2.080+/0.117
Na^{+}	mEq/L	172.200+/-13.348	168.441+/-14.227	175.275+/-14.625	175.337+/-15.738	169.500+/-19.065	172.700+/-18.843
K^{+}	mEq/L	8.490+/-1.743	8.046+/-1.670	7.751+/-1.482	9.020+/-1.958	7.200+/-2.590	7.848+/-2.535
K^{+}/Na^{+}	Ratio	0.049	0.047	0.044	0.051	0.042	0.045
Each value represents the mean +/-SE (Standard error of the mean).							
The mean value represents 6 fishes in number for each subgroup.							

Table (3): Effect of the exposure to the molluscicidal concentrations of *A. arvensis* on some serum enzyme activities of *O.niloticus*.

Groups		Controls		Exposure to 50 ppm		Exposure to 100 ppm	
Subgroup / Parameters		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
AST	U/L	63.240+/-20.359	73.600+/-17.744	66.840+/-21.674	75.000+/-22.560	62.200+/-20.243	70.440+/-18.724
ALT	U/L	44.030+/-9.960	46.231+/-10.458	45.130+/-10.209	44.844+/-10.144	42.929+/-9.711	45.537+/-10.301
AP	U/L	4.350+/-0.440	4.416+/-0.006	3.915+/-0.396	4.858+/-0.013	4.458+/-0.451	3.974+/-0.005
Each value represents the mean +/-SE (Standard error of the mean).							
The mean value represents 6 fishes in number for each subgroup.							

signs, no histopathological observations and no statistically significant biochemical and physiological changes on oral administration of the plant dry powder to groups of albino mice for two weeks in comparing with control groups indicating the safety of this plant to non target animals.

It could be concluded that on comparing and applying the present results of this research with those of previous studies on effects of other plant molluscicides on some non-target livings, *A. arvensis* could be regarded as safe plant molluscicide for *O. niloticus*.

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