Vet.Med.J.,Giza. Vol.48, No.4. (2000) : 529-538.

STUDIES ON THE EFFECT OF THE MOLLUSCICIDAL ACTIVITY OF THE WILD MEDICINAL PLANT *AMMI MAJUS* (UMBILLIFERAE) ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF THE NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Received: 9.4.2000.
Accepted: 25.7.2000.

SUMMARY

Ammi majus (*A.majus*) fruit extract in concentration of 166 ppm and 234 ppm as LC50 and LC99,99 was previously succeed as effective molluscicide against schistosoma snails . The present study aimed at testing the effect of this molluscicide doses on some physiological functions of the Nile tilapia (Oreochromis niloticus=O. niloticus) to show whether this extract is safe and non toxic for these fishes or not . O. niloticus were brought from Bahr Muess at Zagazig City and reared in the laboratory had no clinical signs or abnormal changes externally and in the internal organs of the fish after exposure to the two molluscicidal concentrations for 24, 48 and 72 h . The biochemical results are indicated that the levels of: glucose, total protein, uric acid, urea, aminotransferases (aspartate aminotransferase=AST and alanine aminotransferase = ALT), electrolytes (Na+, K+, Ca++ and P++) and hormones (Triiodothyronine =T3, Tetraiodothyronine =thyroxine =T4 and cortisol) in the serum of the exposed Nile tilapia show no significant alterations after the three exposure times in comparing with controls the non exposed fishes . The study concluded that A. majus fruit extract at their molluscicidal doses has no hazard effect on O. niloticus at the condition of the present study . Further study will be don on different types of the Nile fishes of different ages before commercial application of the new molluscicide .

INTRODUCTION

In snail control programs in some developing countries , the eradication policy are reluctant to embark on synthetic chemical molluscicides for their high cost (WHO, 1982) . Therefore , increas-

ing interest in the plants which are lethal to the intermediat host of schistosoma suggesting that the possibility of available way for control and destruction of snails with little expense, effective , natural and non pollutant in comparing with those of synthetic and organic molluscicides (Msangi and Zeller ,1965; Daffala and Amin ,1976) . In the same respect , the work done by Schonberg and Latif (1954) , Rizk (1995) , Rizk (1999) , Al-Sharkawi (1996 a) and Al-Sharkawi et al . (1996) on A. majus extract proved its marked molluscicidal activity where it shows severe toxicity against snails of schistosoma mansoni, Biomphalaria alexandrina (B. alexandrina) at dose of 166 ppm for 72 h . This molluscicidal activity was attributed to furocoumarin compounds (xanthotoxin, imperatorin and pergapten) which are present in different parts of A. majus plants mainly fruits (Pathak et al., 1962; Lewis and Elven Lewis, 1977) . The active principles furocoumarins of A. majus are considered to be active as sodium pentachlorophenate (NaPCP) which was one of the standard molluscicide of the 1950s as claimed by Abdulla et al. (1977) in Egypt .

A. majus occurs throughout the Nile Valley and Delta where it grows wildly as an annual herb of wide distribution and it has the vernacular name Khilla Shaitani (Boulos and El-Hadidi, 1994) . It is considered as one of the most important Egyptian medical plants where they rich with furocoumarins which have pharmaceutical value as a strong photosensitizing agent extensively used in treatment of leucoderma (Couperus, 1954; Haggag and Hilal, 1977) . Also, Rizk (1995) found that A. majus extract has marked molluscicidal activity against B. glabrata . In addition , the molluscicidal, larvicidal, and meracidal activities of A. majus water extract under direct sunlight showed far higher potency in the control of schistosomiasis (Al-Sharkawi, 1996 b) .

Synthetic chemical and organic molluscicides cause environmental pollution indicated by hazard physiological, biochemical, histopathological and residual changes in different fish species including O. niloticus (Seddek et al. ,1992; Pelgrom et al., 1995; Ghazaly and Said, 1995; Abd El-Aziz et al., 1997) . Therefore, many workers do efforts to overcome the problem of pollution in addition to produce suitable molluscicide for control and destruction of schistosoma snails . Therefore , the aim of the present study is to clarify the effect of A. majus fruit extract in their molluscicidal doses on mature stage of the Nile tilapia and whether it is safe and non toxic or not when this extract is used as molluscicide . This was carried out by measurement of the effect of the molluscicidal concentrations of the plant extract on the levels of some physiological and biochemical parameters in the serum of the exposed O.niloticus.

MATERIALS AND METHODS

Collection of plant materials:-

The best time for collection of A.majus fruits is

in the stage when they become mature, but still unripe, 26 days old to achieve highest yield of furocoumarins (xanthotoxin, bergapten, imperatorin) and mean while reduce the loss in crop to minimum due to the easy dispersion of the fruits (Balbaa et al., 1972 b) . The fruits which are constituting the whole umpel are collected at the early green fruiting stage of flowering season after blooming during May 1999 from the indigenous wild plant vegetation which grown widely in the fields at El- Shobak village near Zagazig city, where cultivation was carried out on October and plants started to give flowers on March. The choice of this plant part is based on the abundance of active principles relative to the other vegetative parts. The fruits are air dried in the shade, then finely blended using an electric laboratory mill. Then, fruit powder is kept in a glass container and stored at the room temperature until use .

Preparation of the extract:-

A stock of isotonic extract is prepared according to Al-Sharkawi (1996a) by soaking ten grams of the powdered fruits in 250 ml distilled water . The solution is warmed at 100°C for half hour in a water bath, then, left at room temperature over night . The result marc is separated by filtration through filter paper and the filtered extract is completed to one liter with distilled water . This hot water extract of A. majus fruit previously proved that has marked molluscicidal activity against B. alexendrina snails as LC50 value of 166ppm and complet death LC99.99 value of 234ppm after an exposure period of 72 h (Al-Sharkawi et al. ,1996) . The working concentrations are freshly prepared from the stock filrate which is always discarded after one week from its preparation time to avoid deterioration .

Fish:-

Adult Nile tilapia (*O. niloticus*) 0f both sexes weighing 100-150 g/ individual are used in this study and they are brought from Bahr Muess as a branch of the Nile River at Zagazig city , Egypt . These fishes are immediately transported to working place under water surface in aerated plastic tanks avoiding agitation, higher temperature and prolonged period of transportation to minimize and reduce stress effect, asphyxiation and mortality resulting from transport . Fishes are divided into 3 equal groups as the first (control), the second (exposed to 166 ppm of the extract) and the third (exposed to 234 ppm of the extract) groups respectively . Each group is subdivided into 3 equal subgroups, where 6 fishes are collected from each subgroup at sampling . Each subgroup is kept in plastic tank of 250 liters capacity and fishes are acclimatized for 15 days for laboratory conditions before exposure to the extract .

According to Stoskopf (1993) at the beginning of the acclimatization period, fishes are subjected to copper sulphate solution bath as 3 mg/L for one hour to stop fungal and parasitic activities . Also , fishes are subjected to a prophylactic doses of

chloramphenicol as l g/10 liters for 48 hours to prevent bacterial infections especially after transportation. Each tank containing fishes are supplied with dechlorinated tap water (after evaporation of chlor) and an air pump for aeration allover the period of acclimatization and period of exposure to the extract. Fishes are kept at feeding commercial ration in the form of artifacial pellets, except 24 hours before collection of samples , feeding is stopped . Fishes in the second and third groups are exposed to the freshly prepared A. majus fruit extract at concentrations of 166 ppm and 234 ppm for each group respectively . Then, blood samples are collected from fishes (from caudal vein) after 24, 48 and 72 hours of exposure. Serum is separated by centrifugation at 3000 rpm for 15 min and kept in vials under deep freezing at -20°C until analysis.

Biochemical assays:

By means of reagent kits, serum levels of glucose, total protein, uric acid, urea and electrolytes Ca++ and P++ are estimated according to: Trinder (1969) , Doumas et al. (1981) , Barham and Trinder (1972) and Fawcett , Scott (1960) and Weissman and Pilleggi (1974) and Tietz (1970)respectively, while, Na+ and K+ are measured by using flame photometre according to Varley et al. (1980) . Also, AST and ALT are determined colormetrically according to Schmidt and Schmidt (1963) . Serum T3, T4 and cortisol levels are measured in Centeral Lab for Assays Services of Nuclear Energy Agency according to Tietz (1995), Albertini and Ekins (1982) and Burtis and Ashwood (1994) respectively.

Statistical analysis: This was applied by using students t-test according to Scendcor and Cochran (1967).

RESULTS

Exposure of O. niloticus for A. majus fruit extract at concentrations of 166 ppm and 234 ppm for 24, 48 and 72 hours show no any clinical sings or abnormal changes externally and in the internal organs of the exposed Nile tilapia in response to these molluscicidal concentrations of A. majus .

As shown in tables (1), (2) and (3) there are no significant changes in different estimated parameters in the serum of O. niloticus on response to exposure for A. majus fruit extract at concentrations of 166 ppm and 234 ppm for 24, 48 and 72 hours . This cleared that the serum levels of glucose, total protein, uric acid, urea, aminotransferase activities (AST and ALT), electrolytes (Na+, K+, Ca++ and P++) and hormones (T3, T4 and cortisol) of O. niloticus are not affected as a result of exposure to A. majus fruit extract in their concentrations that previously kill the exposed schistosoma snail hosts .

Table (1): Effect of A.majus fruit extract exposure on some serum parameters of O.niloticus.

Parameters	Periods of exposure								
	24 hours			48 hours			72 hours		
	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm
Protein g/dl	3.900± 0.451	4.300± 0.333	4.450± 0.806	3.833± 0.463	4.000± 0.419	3.720± 0.492	3.650± 0.356	4.100± 0.307	4.250± 0.891
Glucose mg/dl	114.500± 15.950	111.833± 11.703	120.500± 11.811	121.000± 15.760	127.333± 15.028	128.333± 16.966	118.200± 18.225	107.200± 15.548	102.300± 12.323
Uric Acid mg/dl	2.050± 0.339	2.13± 0.432	2.120± 0.271	2.150± 0.225	2.300± 0.442	2.200± 0.374	1.800± 0.394	1.850± 0.488	1.830± 0.398
Urea mg/dl	12.200± 3.544	12.700± 4.501	13.000± 3.898	14.700± 3.204	15.000± 5.477	16.333± 4.676	13.200± 4.833	11.700± 4.676	13.000± 5.366
AST U/L	52.7± 16.966	55.700± 18.062	51.833± 16.869	61.333± 14.787	62.500± 18.800	58.700± 15.603	58.000± 16.284	59.700± 21.685	54.200± 14.121
ALT U/L	8.333± 2.943	9.500± 3.082	8.500± 2.738	10.333± 2.943	13.700± 3.386	11.700± 2.160	9.700± 3.700	10.333± 3.333	9.333± 3.011

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 A.majus fruit extract exposure on some serum hormones of O.niloticus.

Parameters	Periods of exposure								
	24 hours			48 hours			72 hours		
	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm
Na mEq/L	168.000± 13.023	171.000± 14.269	169.500± 19.065	164.333± 13.880	179.833± 16.142	172.700± 18.843	172.700± 11.052	176.200± 10.381	163.500± 18.415
K+ mEq/L	8.283± 1.701	7.200± 2.590	7.950± 1.521	7.850± 1.630	8.050± 2.601	9.020± 1.958	7.850± 1.469	6.800± 2.600	7.633± 1.478
Ca++ mg/dl	15.588± 1.724	15.420± 1.079	15.283± 1.150	14.720± 1.538	16.600± 1.017	16.950± 1.767	14.620± 1.543	14.820± 1.404	14.320± 1.399
P++ mg/dl	15.350± 1.630	16.050± 1.630	15.433± 2.702	14.333± 1.722	17.233± 2.577	16.600± 2.995	14.320± 1.373	14.933± 1.949	14.000± 3.069

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 A.majus fruit extract exposure on some serum electrolytes of O.niloticus.

Parameters	Periods of exposure								
	24 hours			48 hours			72 hours		
	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm	Controls	166 ppm	234 ppm
T3 ng /dl	140.500± 20.945	150.200± 20.701	135.000± 20.000	136.200± 22.851	155.833± 24.045	145.500± 22.256	134.000± 20.350	160.500± 20.255	128.700± 17.500
T4 ng /dl	0.253± 0.038	0.272± 0.047	0.283± 0.027	0.223± 0.031	0.263± 0.036	0.273± 0.043	0.228± 0.040	0.238± 0.062	0.243± 0.040
Cortisol ug /dl	13.800± 3.357	13.700± 3.104	13.100± 3.3216	13.000± 3.373	15.083± 3.276	14.183± 3.130	13.400± 3.479	12.833± 3.109	12.550± 3.614

Each value represents the mean ± SE (standard error of the mean).
The mean value represents 6 fishes in number for each subgroup.

DISCUSSION

So far as the authors are aware, there is no references concerning the effect of A. majus extract on biochemical and physiological status of fish species . Therefore, this work may be considered as the first record for this investigation of the Nile tilapia response to exposure to the molluscicidal concentrations of A . majus fruit extract .

As shown in this study, the obtained data reflect no marked response of O. niloticus to the presence of the tested plant extract, where, there are no clinical sings or abnormal changes externally and internally on fish . Also, there are no significant deviations in any measured parameters in the serum of the exposed fish . This means that the concentrations of the extract which are used as molluscicide may not be stress, toxic, anoxious or potential action on the Nile tilapia .

This end result is in harmony with the conclusion of El-Bolkiny et al. (1997) who studied the effect of the isotonic extract of A. majus at different concentrations on the stability of RBCs of human, sheep, buffalo, rat, duck and catfish . However, the authors found that the effective haemolytic concentrations are very higher than the proposed molluscicidal LC50 of the extract for different studied RBCs . They also reported that the in vitro significant effective haemolytic concentration of A. majus extract on RBCs of catfish (Clarias lazera) is 3500 ppm.

In the present study, the choice of the measured parameters is applied according to the sensitivity of these parameters to response for any stress action environmentally around the fish in its ecosystem . Blood glucose appears to be a typical sensitive reliable indicator of environmental stress in fish (Chavin and Young, 1970; Hattingh, 1976) .

Proteins are involved in the architecture, physiology and metabolism of the cell (Mommsen and Walsh, 1992), while, their synthesis and secretion are attributed to liver efficiency (Alvan, 1986).

As well, urea and uric acid are byproducts of protein catabolism and excreted via kidney as their levels in serum of fish indicate to renal function and overload in the kidney (Ander and Roger, 1996). Also, Kaneko (1980) reported that the rate of urea formation depends on the rate of protein catabolism and Maite et al. (1984) recorded that uric acid can be used as rough index of the glomerular filtration rate . In addition, determination of AST and ALT have been proved to be useful in diagnosis of diseases and affections of liver and kidney of fish (Pickering, 1981; Sandnes et al., 1988) leading to cell membrane damage to become more permeable for huge enzyme quantities to the extracellular fluid and serum (D`Apollonia and Anderson, 1980) .

Serum electrolytes play an important role in the ionic balance of the cell and stress changes in osmoregulation of gills in fish, where, these changes are manifested by altered plasma ion concentrations (Heath, 1987; Abu El-Ella, 1996) . Consequently, ionic equilibrum in the serum of the fish means no gill impairment and not stressed .

Serum titres of cortisol and thyroid hormones are considered in stress and mediated changes in fish (Brown et al., 1978 ; Barton et al 1980 ; Leatherland, 1985) . Also synthesis and release of cortisol is an indicator of toxicity in fish (Donaldson and Dye, 1975; Shenouda et al., 1994) and of stress and stress levels (Leatherland and Sonstegard, 1984; Fatma et al., 1997) in teleostean fish and in male Tilapia nilotica respectively . However, cortisol influences an arry of physiological parameters including carbohydrate, hydromineral balance, mobilization of animo and fatty acids from cellular stores , gluconeogenesis and plasma protein production (Goss and Wood , 1988) .

In the present study, the obtained data show that the serum of the Nile tilapia present no significant alterations in the levels of glucose, total protein, urea, uric acid, AST, ALT, electrolytes (Na+, K+, Ca++ and P++) and hormones (T3, T4 and cortisol). In addition, there are no clinical signs and no abnormal changes externally or internally on different vital organs of fish (Liver, Kidney, Gills) after exposure to the molluscicidal concentrations of A. majus fruit extract for 24, 48 and 72 h . On comparing and applying these present results with those previously shown by all above previous authors indicated the safety and non toxicity of A. majus fruit extract at molluscicidal concentrations for O. niloticus in freshwater ecosystem and its availability, cheapness and effectiveness . Therefore, this work opens a new field to apply this plant extract in freshwater ecosystem for control of schistosomiasis instead of synthetic, chemical and organic molluscicides which are pollutants and hazard for fish and human being after further

study on other different fish species of different ages and conditions .

ACKNOWLEDGEMENT

We thank for Dr. R.M.Khodary, Researcher of Pharmacology for assistence of collection of A. majus and preparation of its extract.

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