Veterinary Medical Journal-Giza (ISSN 1110-1423)
Faculty of Veterinary Medicine,Cairo University
Accredited from national authority for Quality Assurance and Accreditation
Giza, 12211, Egypt

Assessment of toxic effects of aqueous extract of crude Desert date(*Balanitus aegyptiaca*) stem bark on the general health status of *Orechromis niloticus*

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Abstract

The following studies were designed to evaluate the toxic effect of the aqueous extract of *Balanitus aegyptiaca* on O.niloticus general heath status.300 *O.niloticus* were used to determine the acute half lethal concentration (LC50/96hrs) of the aqueous extract of *Balanitus aegyptiaca* . Results revealed that the (LC50/96hrs) in *O.niloticus* was 69 ppm.The effects of short term exposure to 1/2 (LC50/96hrs) for one week revealed some clinical abnormalites in the form of abnormal swimming behavire, respiratory distress with suffocation, fins rot and congested. gills.The postmortum examination revealed congestion and enlargement of liver. The effects of long term exposure of *O.niloticus* to to 1/10(LC50/96hrs) for 8 weeks revealed clinical abnormalites in the form of respiratory distress with congested. gills in some fishs. Hematological examination during the short term and long term exposure of *O.niloticus* revealed that the RBCs count, PCV and Hb were significatly increased espicially in long term exposure.Leukocyte count was increased significantly only in short term exposure comparing with the control group. Serum enzymes levels of *O.niloticus* in both short and long term exposures showed high levels of ALT and AST than the control group, while there were no significants changes in Urea and Creatinine levels.Histopathological iexamination of fish exposed to ½ and 1/10 of (LC50/ 96) showed congention and hyperplasia of secondary gill lamellae in few cases, congestion and dilatation of hepatoportal blood vessels with swelling of hepatocytes. Resudiual analysis of *O.niloticus* exposed to 1/10 (LC50/96h) for 8 weeks were 3.918 ± 0.12 mg/kg of *O.niloticus* muscle. *O.niloticus* when challanged with A.hydrophila after 8 weeks of exposure showed lower mortality rate than (-) control non exposed group. By all means, it appear that water pollution by chemical from plants extract in case of illegall fishing is a disaster to fish population. It leads to severe ecnomic losses through fish mortalities,poor production and higher susceptibility to different fish diseases as a result of severe stress.

key words:- Orechromis niloticus, Balanitus aegypticus, heamatological parameters,histopathological investigation,lysozyme activity assay ,Aeromonas hydrophila

Introduction

It is widely accepted that there is a severe problem with future global food security and large number of the world's fish stocks are currently depleted,due to illegal and unreported fishing. It lead to a loss of many billions of dollars of annual economic benefits creates significant environmental damage through the use of unsustainable fishing practices and has wider consequences for food supply **(Agnew *et al.*,.2009).**

Diffrent ways and materials are used in illegall fishing in natural resources including using of chemicals like potassium or sodium cyanide,explosives,electeric currents,gases or using unsuitablr nets. Plants have long been used by mankind as a source of active compounds for a whole range of activities. Macerated material of plant origin is thrown into rivers, streams or shallow ponds, and then fish stupefied and float to the surface of water and can be easly collected. Subsequent chemical analysis of plant material has revealed that the active compounds in such plant extracts is saponin, but information about the mode of action is relatively little about the saponin (**Neuwinger,2004**)

Balanites aegyptiaca is one of the most widely distributed trees in the dry-lands of Africa and Asia, a small to medium-sized dryland tree or shrub belonging to family

Zygophyllaceae. It makes up to one third of the total tree population in central region of the Sudan and as a multi-purpose tree, *B.aegyptiaca* provides food, medicines, cosmetics, fodder, fuel wood and pesticides. It is commonly known as desert date containing steriodal saponin. *B. aegyptiaca* fruits and stem bark were used for illegall fishing by local citizens because of their efficacy, effectiveness, low cost and lack of fishing gear and net for illegall fishing. **(NRC,2008).**

The physical and chemical changes in aquatic environment due to illegall using of such chemicals and plants often cause negative drastic impact on fish stocks,thus the water quality of an aquatic body is very crucial because it determine the productivity and other parameters which necessary for the fish survival.**(Fafioye, 2001).**

In Sudan, the available information about the diffrenent effects of *B. aegyptiaca* extract on the general health status of freshwater fish are little, so the aim of this study is to investigate different toxic effets of this material on *Orechromis niloticus* through declaring of the 96 (LC50), clinical signs,P/M lesions after acute and chronic exposure as well as determinig hematological, biochemical,histopathological and immunological changes as well as residual analysis in order to evalute the public health significance.

Materials and Methods

2-1 Plant material

2-1-1 Plant prepration : *Balanites aegyptiaca* stem bark were collected in June from Khartoum state,Sudan and authenticated in Botany Department, University of Khartoum. It was washed with water and then dried in air and sun for fifteen days, T followed by drying in the oven for 24 hours.The dried materials was grinded to fine powders and placed in a dry plastic container prior to extraction.

2-1-2 Phytochemical screening: Phytochemical analysis for the crude stem bark of *Balanites aegyptiaca* was carried out using standard laboratory methods and standardized chemical tests. It revealed the presence of the following active ingredients: saponins, tannins, flavonoids, steroids and alkaloids. The stem bark powder was pale yellow with bitter taste and the odour is irritating to mucous membrane of the nostrils. Inorganic minerals namly (Ca,Fe,P,S,Mn and K) were present according to **Gupta *et al.* (2012).**

2-2 Expermintal fish: Total of 300 cultured *O.niloticus* healthy fish with average body weight (90 ± 10) gm and length (17 ± 2) cm, were collected in novmber 2013, from ponds of the World Fish Center at Abbassa, Abo-Hammad,Sharkia, Egypt. Fish were acclimated in glass aquaria for two weeks filled with dechlorinated tap-water supplied with continuous aeration prior to the experiments. The fish were randomly stocked at a rate of 10 fish per aquarium. The temperature was kept at 26 ± 2 °c throughout the experiment. Fish were fed twice daily with standard commercially prepared pellets at 3% of the body weight allover the experimental period.

2-3 Experimental design:-

Experiment A : (Determination of (LC50/96) to *O. niloticus*.

One hundred and twenty of *O.niloticus* were used to determine the acute half lethal concentration for 96h. A minimum of five concentrations (three replicates per concentration); namely: 50,60,70,80 and 90 mg/L plus three control were applied. Thirty fish were used for each concentration divided in three aquariua filled with dechlorinated water and supplied with oxygen from electric water pumps. The LC50/96 was calculated

according to method of **Litchfield and wilcoxon (1949).**

Experiment B : (short term exposure or acute toxicity):

30 *Oreochromis niloticus* were divided in 3 aquaria where 10 fish kept in each aquaria. The two sub groups exposed to 1/2 (LC50/96) of the aqueous extract of Desert date for 7 days and the third one acted as a control .

Experiment c : (Long term exposure or chronic toxicity)

50 *Oreochromis niloticus* were divided in 5 aquaria where 10 fish kept in each aquaria. The four sub groups exposed to 1/10 (LC50/96) of aqueous extract of Desert Date for 8 weeks and the fifth one acted as control.

2-4- Clinical signs and post-mortem findings

Clinical examination of fish was carried out according to the method described by(**Amlacher, 1970; Austin and Austin, 2007).** Postmortem examination of fish was performed on moribund sacrificed fish and/or freshly dead fish according to the method described by **(Conroy and Herman 1981).**

2-5- Hematological investigations

Blood samples were taken from caudal vessels of experimental *Oreochromis niloticus* after one week by syringe using heparin as anticoagulat (1000 unit/ml blood), from both control and exposed groups afterr 7 days for acute toxicity and after each two weeks (2nd,4th, 6th and 8th week) for chronic toxicity. Blood was used for determination of different blood parameters (erythroyctic count, and leucocytic count according to **(Dacie & Lewis,1984)**,while Hemoglobin concentration estimated according to **Jain(1986)** and PCV was calculated according to the formula mentioned by (**Dacie & Lewis, 1984**).

2-6- Serum Biochemical analysis

Blood was collected in plain centrifuge tubes; centrifugated at 3000 r.p.m. for 15 minutes for serum separation for determination of serum transaminases(ALT and AST) from both control and exposed groups afterr 7 days for acute toxicity and during (the 4th and 8th weeks) for chronic toxicity according to **Reitman and Frankel (1957),** while serum creatinine measured colorimetrically according to **Henry (1974)** and serum uric acid measured by using enzymatic determination method **Barham and Trinder (1972).**

2-7-Histopathological examination

Specimens from the liver and gills were collected from both control and exposed fish after one week for acute toxicity. The same samples were taken after 8 weeks for chronic toxicity for microscopic changes. The collected Tissue specimens were fixed in 10% buffered formalin solution according to **(Culling, 1983).**

2-8 Residual analysis

Muscle samples (5 g dry wt from muscle) from ten *Oreochromis niloticus* exposed to 1/10 (96 LC50) of aqueous extract of Desert Date for 8 weeks were took and kept frozen at -20°C till analysis by High-performance liquid chromatography (HPLC) for residual according to (**Kostova and Dinchev, 2005).**

2-9- Evaluation of lyzozymes acivity

Oreochromis niloticus exposed to 1/10 (96 LC50) of aqueous extract of Desert Date for 8 weeks, Experimentally infected by an intraperitoneal injection of *A. hydrophila.* The challenged bacterium was obtained as a reference pathogenic *Aermonas hydrophila* strain that was previously isolated from the liver of morbid O. niloticus and tested for pathogenicity in the World Fish Center (Egypt). A suspension of *A. hydrophila* was prepared by culturing in Tryptic soy agar for 24 h/at 37 ℃ . The broth culture was centrifuged at 3000 g for 10min. The supernatants were discarded and the pellets were re-suspended in phosphate-buffered

saline (PBS 7.4), and the OD of the solution was adjusted to 0.5 at 456 nm, which corresponded to 1 X10^9 cells/ ml (**Sahu *et al.*, 2007**). This bacterial suspension was serially diluted using standard dilution technique with PBS and used for the challenge experiment and bactericidal, then collected, washed, and suspended in sterile saline 0.85% and counted using McFarland standard tubes. The mortality was monitored and recorded daily for 7 days. The percent was calculated. Freshly dead fish were cultured for the presence of A. hydrophila to confirm cause of mortality using standard microbiological procedures according to **(De-Hai *et al.*, 2012).**

Group of fish	No. of fish	Exposed /or not	Injected by
1st aquaria (A1)	10	Exposed	Saline
2nd aquaria (B1)	10	Exposed	*A.hydrophila*
3rd aquaria (A2)	10	Not exposed	Saline
4rd aquaria (B2)	10	Not exposed	*A.hydrophila*

Statistical analysis

The data obtained in this study were statistically analysed using analysis of SPSS (Independent sample test) according to **Snedecor and Cochran (1969)** for comparing the different mean values with Duncan´s multiple range test by Duncan´s.

Results

Determination of 96 (LC50 to Tilapia and Catfish):

The (LC50/96) was calculated according to method of **Litchfield and Wilcox,(1949)** and was founded 69ppm.

Acute and chronic toxicity tests Clinical signs and post-mortem findings

In the short term exposure to 1/2 (LC50/96) (34.5 ppm) of aqueous extract of Desert date to *Oreochromis niloticus* the clinical signs was manifested in the form of abnormal swimming behavior in form of avoiding and scaping espiecially at the start of exposure, swimming laterally calm till death, still breathing but apparently paralyzed espiecially before death,also the fish showed respiratory distress characterized by surface swimming and gasping in addition to skin scales detachment, dark colouration of skin, hemorrage and severe fins erosion as showen in (figure 1 A and B). The Post-mortem examination revealed congested gills ,enlargment and congestion in liver as showen in (Figuer 2 A,B,C and D).

In the long term exposure to 1/10 (LC50/96) (6.9 ppm) of aqueous extract of Desert date to *Oreochromis niloticus*, the fish appeared clinically normal and the above-mentioned clinical signs and post-mortem finding were not observed except of the enlargement and congestion of liver.

Blood parameters

In the present study, the erthrocyte count, haemoglobin,PCV value were significantly increased in both acute and chronic exposed groups of *O.niloticus* in comparison with the control non exposed group, while there was no significant change in the mean values of MCV,MCH and MCHC in both acute and chronic exposed groups of *O.niloticus* in comparison with the control non exposed group. The WBC count was significantly increased in the short term toxicity, while there was no change in the long term toxicity as shown in table (1) and table(2).

Table 1. Effect of aqueous extract of *Balanitus aegypticus* on blood parameters of *O.niloticus* after one week (Mean ± SE).

Paramerters	Control	Acute term toxicity
RBC(10^6/mm^3)	1.820 ± 0.02	2.160 ± 0.16
Hb (g/ 100 ml)	4.140±0.250	4.580 ± 0.25*
PCV %	17.140 ± 0.33	20.50 ± 0.74*
WBC(10^3/mm^3)	14.14 ± 0.60	19.66 ± 0.40*
MCV (fentolitre)	94.00 ± 0.37	94.17 ± 0.83
MCH (Pg/cell)	22.72 ± 0.90	21.58 ± 0.91
MCHC (gm/100ml)	22.66 ± 0.56	22.45± 0.86

*Means are significant at P≤0.05 level .

Table 2. Effect of aqueous extract of *Balanitus aegypticus* on blood parameters of *O.niloticus* after (2nd, 4th, 6th and 8th weeks) (Mean ± SE).

Paramerters	Control	Acute term toxicity
RBC(10^6/mm^3)	1.820 ± 0.02	2.160 ± 0.16
Hb (g/ 100 ml)	4.140±0.250	4.580 ± 0.25*
PCV %	17.140 ± 0.33	20.50 ± 0.74*
WBC(10^3/mm^3)	14.14 ± 0.60	19.66 ± 0.40*
MCV (fentolitre)	94.00 ± 0.37	94.17 ± 0.83
MCH (Pg/cell)	22.72 ± 0.90	21.58 ± 0.91
MCHC (gm/100ml)	22.66 ± 0.56	22.45± 0.86

*Means are significant at P≤0.05 level .

Biochemical profile

There was significant increase in the mean values of ALT and AST in both acute and chronic exposed groups of *O.niloticus* in comparison with the control non exposed group ,while there were no significant changes in the levels of (Uric acid and Creatinine) in both acute and chronic exposed groups of *O.niloticus* in comparison with control non exposed group.

Table 3 Effect of aqueous extract of *Balanitus aegyptiaca* on liver and kidney function of *Orechromis niloticus* after 7 days of exposure (Mean ± SE).

Paramerters	Control	Acute term toxicity
ALT(mL/L)	27.94 ± 1.77	74.20 ± 6.12*
AST(mL/L)	28.18 ± 2.26	67.60 ± 8.08*
Uric acid (mg/dL)	1.98 ± 0.14	1.97± 0.10
Creatinine (mg/dL)	0.236 ± 0.21	0.25 ± 0.31

- Means are significant at P≤0.05.

Table 4. Effect of aqueous extract of *Balanitus aegyptiaca* on liver and kidney function of *O.niloticus* after (4th ,8th) weeks of exposure (Mean ± SE).

Paramerters	Control	4 weeks	8 weeks
ALT(mL/L)	23.52 ± 1.14	30.10 ± 1.70*	42.80 ± 4.53*
AST(mL/L)	21.17 ± 1.20	34.26 ± 4.81*	38.40 ± 4.65*
Uric acid(mg/dL)	2.14 ± 0.12	2.21 ± 0.78	2.16 ± 0.13
Creatinin(mg/dL)	0.246 ± 0.21	0.232 ± 0.53	0.221 ± 0.31

- Means are significant at P≤0.05

Histopathological investigation:

Gills: Microscopic examination of the gills of *O.niloticus* showed hyperplasia,hypertrophy and fusion of secondary gill lamellae as illustrated in figure (3 A) .

Liver: Microscopic examination of liver showed Heavy new vascularization, congestion with hemolysis of hepatoportal blood vessels, hemorrhage and vacoulation of hepatocytes as illustrated in figure (3 B).

Residual analysis

In this study, concentratiion of residues were determined from muscle of 10 exposed *Orechromis niloticus* for 8 weeks intoxication, analysis releaved (3.918 ± 0.12 mg/Kg), so it had high capacity for bioaccumulation in the muscles.

Lysozyme activity assay: The percentage of mortality rates were decreased in the exposed *Orechromis niloticus* to Desert date for 8 weeks in comparison with (-) control non exposed group when challenged with *A.hydrophila* .

Table (5) showed the percentage of mortality rates and lysozyme activity of the exposed*O.niloticus* to desert date for 8 weeks and non exposed *O.niloticus* when challenged with *A.hydrophila*

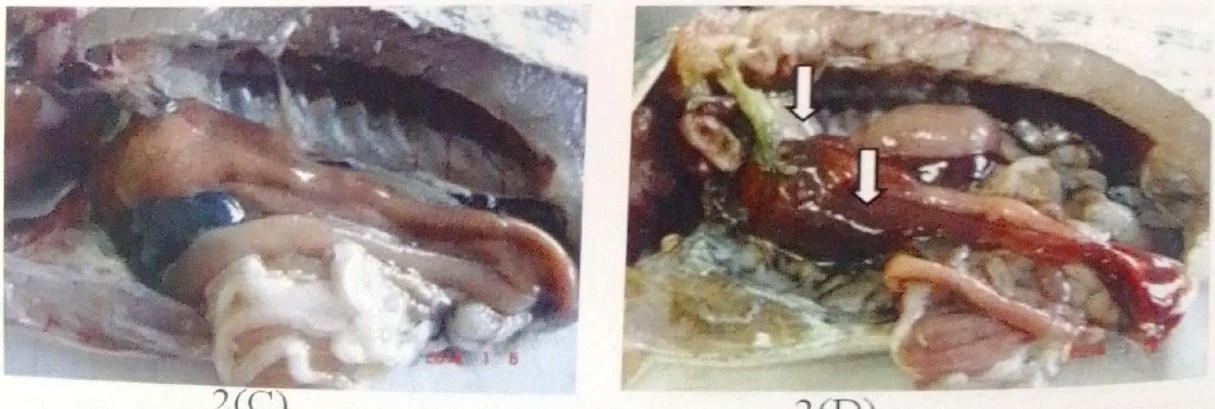
Group of fish	No of fish	Exposed/or not	Injected by	Mortality No.	Mortality %	Lysozyme activity
1st aquaria (A1)	10	Exposed	Saline	0	0	13.20 ± 1.20
2ndaquaria (B1)	10	Exposed	*A.hydrophila*	2	20	10.40 ± 1.04
3rdaquaria (A2)	10	Not exposed	Saline	0	0	8.20 ± 0.96
4thaquaria (B2)	10	Not exposed	*A.hydrophila*	8	80	6.20 ± 0.51

1(A) 1(B)

Fig 1 (A,B) showing Clinical signs of acutely exposed *Orechromis niloticus*
A. Acutely exposed *O niloticus* showing showing scales detachment and dark colouration of the skin.
B. Acutely exposed *Orechromis niloticus* showing tail fin erosion,sloughing and haemorrage

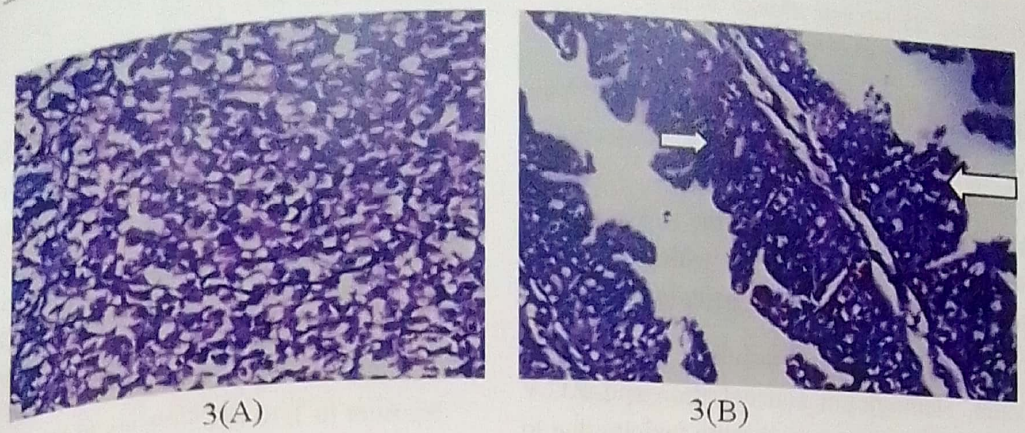
2(A) 2(B)

Fig (2 A and B) showing acutely exposed *Orechromis niloticus with* congested gills



2(C) 2(D)

Fig (2 C and D) showing acutely exposed *Orechromis niloticus with* enlargment and congestion in liver



3(A) 3(B)

Figure 3(A) Liver of *Orechromis niloticus* showing vacoulation of hepatocytes (H&E stain X1000)
Figure 3(B) gill of acutely exposed *Orechromis niloticus* showing hyperplasia,hypertrophy(large arrows) and fusion of secondary gill lamellae (small arrow)

Discussion

Illegal fishing creates significant collateral damage to ecosystems. contributing to over exploitation of stocks, The shortage of fish production from the natural water resources apart due to the water pollution and also due to the illegal fishing,directed us to fish culture. The fact that fish can use the water and not exhaust the water is stimulus to initate different fish culture systems (**Agnew *et al.*, 2009**).

In Sudan, B. aegyptiaca stem bark aqueous extract used in illegall fishing in the west of sudan (AL Radoum) but the available information about the diffrenent effects of B. aegyptiaca extract on the general health status of freshwater fish are little, so the aim of this study is to investigate different toxic effets of this material on *Orechromis niloticus* .

The aqueous extract of *Balanitus aegypticus* have toxic effects on *Orechromis niloticus* and the result showed that the (LC50/96h) were 69mg/L in *Orechromis niloticus*,which was not similar to the limits of the study of (**Okwuosa *et al.*, 1993**) and this may be due to the diffrenent in the concentration of the saponin in parts of the plant,also due to the diffrenet in the age and fish species.

No such mortality was recorded in the control experiment during the long and short term toxicity and this is an indication that toxicity was dose-dependent and varies within the time of exposure of aquatic organisms to toxicants and this agree with **(Ayoola *et al.*, 2011 and Fafioye, 2001)** findings.

Fish take saponins directly into their bloodstream through their gills. By changing a large number of behavioural response in the short and long term of exposure, fishes try to resist the change in aquatic environment and reduce the harmful effect of stem bark extracts. By increasing mucous secretion, fishes try to reduce their entrance through body surface or may to reduce absorption of the toxicants and the clinical symptoms recorded during our study were manifested by respiratory disorders in fish. This could be attributed to the saponin nature as a respiratory toxicant, causing decrease in oxygen consumption, irritation to gill epithelium leading to excessive amount of mucous secretion which associated with low dissolved oxygen in water due to formation of foam during solubility of plant extract in water causing suffocation and death,this agree with **(Ufodike and Omoregie 1994; Cagauan *et al.*, 2004**; **Omoniyi *et al.* 2002; Fafioye *et al.*, 2004; Aguigwo 1998 and Adewoye,2010).** Symptoms of toxicosis observed in fish behaviourl include lack of balance; air gulping, restlessness, sudden quick movement, excessive secretion of mucus, settled at the bottom, and died.This

mucus, settled at the bottom, and died.This agree with symptoms of toxicosis observed in fish behaviourl with glycosides. **(Ayoola, 2008).** The skin lesion in the present work may be attributed to saponin which are potent skin senstizers induce skin irritation

There were increasing in the in RBCs count, Hemoglobin concentration and P.C.V levels in the acute and chronic toxicity due to that steroidal saponins are known to have stimulatory effect on heamopoietic organs resulting in increased erythropoiesis,also it may attributed to the interaction between the saponin and the lipids of cell membrane making small pores in membrane which lead to the increaseing the permeability of the cells leading to improving of cell functions.These results agreement with **(Francis,2002 ;Osman *et al*, 2010 and Furo and Ambali 2012).**

Regarding the biochemical serum analysis results indicated an increase in level of ALT and AST liver enzymes. This increase could be attributed to the injury of hepatocytes during the resistance to the toxin and toxin metabolites as confirmed by the histopathological findings this agree with **(Kew, 2000 and Sudhanshu and Ajay 2004)**.On the other hand no change in serum uric acid and creatinine values in fish in the acute and chronic toxicity this confirmed by the histopathological findings in kidneys which revealed no histopathological changes were found in the acute and chronic exposure experiments this agree with **(Obidah *et al*, 2009 and Adewoye,2010)**. Liver showed heavy new vasculization with heamorragic, edmatous with in the tissue, with dilatation and congested of blood vessels of liver which caues the enlargment of liver and increasig the blood supply cause the congestion in the liver .This is agree with **(Annune& Ajike, 1999; Costa *et al.*, 2009; Ayoola *et al.*, 2008; Adeogun *et al.*, 2012 and Keziah,2013).** Histopathological examination to gills showed congested blood vessels with fusion and hyperplasia of seondary gill lamellae this fusion due to irritant material whether dissolved or suspended in water. This fusion and hyperplasia of secondary gill lamellae reduced the gap between the secondary lamellae and so gas diffusion is impaired and fish showed sings of asphyxia as we noticed in the short term exposure according to(**Annune and Ajike 1999**). Fish 8 weeks post exposure to 1/10 (LC50)96hrs of the aqueous extract of *Balanitus aegypticus* recorded significant decrease in the mortality rate, after challenge with *A.hydrophyla* comparing with control(-) non exposed group and this agree with **(Aly and Fathi 2008)** also the levels of lysozyme activity in the exposed group is more than the non expoed group This indicated that aqueous extract of *Balanitus aegypticus* may have an immunostimulant effect on fish and similar results were reported by **(Kensil, 1996; Bagalwa and Chifundera *et al.*, 2007 and Osman *et al.*, 2010)** studied that saponins interfere with membrane bilayers leading to and enhance penetration of macromolecules such as proteins through cell membranes so they have used as adjuvants in vaccines.

References

Adeogun, A. O. Alaka,O.O. Taiwo,V.O. and Fagade,S. O. (2012): Some Pathological Effects of Sub-lethal concentrations of the Methanolic Extracts of Raphia hookeri on Clarias gariepinus Afr. J. Biomed.,15: 105 – 115.

Adewoye,S.O. (2010): Haematological and biochemical changes in vivo Clarias gariepinus exposed to Trephosia vogelii saponin extract App Sci Res. 1 (1): 74-79

Agnew,D.J.; Pearce, J.; Pramod, G.; Peatman, T. and Watson, R.(2009): Estimating the Worldwide Extent of Illegal Fishing. PLoS ONE 4(2): e4570.

Agwuigwo, J.N (1998): Studies on the acute toxicity of cassava leaf extracts on African catfish, Clarias anguillaris. J. Aquatic Sci. 13: 29-32

Aly, S. M. and Fathi, M.M. (2008): Echinacea purpurea and Allium sativum as immunostimulants in fish culture using Nile tilapia (Oreochromis niloticus) J. of Animal Physiology and Animal Nutrition 94 (2010) e31–e39

Amlacher, E. (1970): Textbook of Fish diseases. T. F. H. Publications, Neatune city, New Jersy: 117-135.

Annune, P.A. and Ajike, S.U. (1999): Acute toxicity and Gill morphology of Oreochromis niloticus (Trewavas).Exposed to Rigor. J. of.Aqu.sci.14: 1-4.

Austin, B. and Austin, D. A. (2007): Bacterial fish pathogens: "Diseases of farmed and wild fish", 4th revised Ed. Springer Praxis Publishing, Chichester, UK

Ayoola, S.O. (2008): Toxicity of Herbicide, Glyphosate on Nile tilapia (Oreochromis niloticus) Juvenile. Afr. J. of. Agricult.res.3(12): pp.825-834.

Ayoola,S.O.; Kuton,M.P.; Idowu,A.A. and Adelekun, A.B.(2011): Acute Toxicity of Nile Tilapia (Oreochromis niloticus) Juveniles Exposed to Aqueous and Ethanolic Extracts of Ipomoea aquatica Leaf. Nat and Sci 9(3):91

Bagalwa, M. and Chifundera, K. (2007): Environmental impact evaluation of the stem bark extract of Maesa lanceolata used in Democratic Republic of Congo Journal of Ethnopharmaco.114,281–284.

Barham, D. and Trinder, P. (1972): Enzymatic determination of uric acid. Analyzed, 97: 142-145.

Caguan, A.G.; Galaites, M.C. and Fajardo, L.J. (2004): Evaluation of botanical piscicides on Nile tilapia Oreochromis niloticus L. And mosquito fish(Gambusia affinis)Baird and Girard Proceedings on ISTA, 12-16 September. Manila, Phillipines: 179-187 .

Conroy, D.A. and Herman, L. R. (1981): Textbook of Fish diseases. T. F. H. Publication, West Sylvania

Costa, P. M.; Diniza, M. S.; Loboa, S. C.; Martinsc,J. M. Ferreirac, A. M.; Caetanoc,M. C.;Valec, T. A. And Costa , M. H.(2009): Histological biomarkers in liver and gills of juvenile Solea senegalensis exposed to contaminated estuarine sediments: Aqua. Toxicol.,92: 202–212.

Culling, C.F. (1983): Handbook of Histopathological and Histochemical techniques, 3rd ed., Butterworth, London.

Dacie, J.V. and Lewis,S.N. (1984): Practical Haematology. 6th Edition Edinburgh, Churchill Livinstone.

De-Hai,X.U. ; Julia,W. P.; Phillip,K.C. and Shoemaker,A. (2012): Parasitism by protozoan Ichthyophthirius multifiliis enhanced invasion of Aeromonas hydrophila in tissues of channel catfish. Vet.Parasitol ., 184 . 101:107.

Fafioye,O.O(2001): Lethal and sub lethal effect of extracts of Parkia biglobosa and Raphia vinifera on some freshwater fauna. J.App.Sci.,1(1): 74-79

Fafioye, O.O.; Adebisi, A.A. and Fagade, S.O. (2004): Toxicity of Parkia biglossa and Raphia vinifera extracts on Clarias gariepinus juveniles. Afri. J.Biotech. 3(11): 627-630.

Francis,G.;Makkar,H.P.S.; Kerem,Z. and Becker,K. (2002): The biological action of saponins: a review. Bri. J.of Nutr.88: 587-605.

Furo ,N.A. and Ambali ,A. G. (2012): An investigation into the phytochemical constituents of Moringa oleifera aqueous bark extracts Centrepoint Journal (Science Edition, 18: pages 1 – 7.

Gupta,S.C.; Shenoy,S. and Kotech,M. (2012): Pharmacognostical and Phytochemical valuation of Balanites aegyptiaca(L.) Del. Stem bark Int.res j. pharm., 3(7):169- 172

Henry, R. J. (1974): Clinical Chemistry Principles and Techniques. 2nd ed., Harper and Row Publ., New York, USA.

Jain,N.C.(1986): Schalms veterinary heamatology 4th edition,Lea and Febiger ,Philadeelphia pp 1221.

Kensil, C.R.(1996): Saponins as vaccine adjuvants. Crit. Rev.Ther. Drug Carrier Syst. 13, 1–55.

Kew.M.L.(2000): Serum aminotransferase concentration as evidence of hepatocellula damage. Lancet .335:951-952.

Keziah, I.N. (2013) : Bioaccumulation and toxic effect of some heavy metals in freshwater fish ARI ., 10(3): 1792 – 1798.

Kostova, J. and D. Dinchev, D.(2005): Saponins in Tribulus terrestris chemistry and bioactivity. Phytochemistry Reviews, 4 (1): 111–137.

Litchfield,J.T. and Wilcoxon,F. (1949): A simplified method of evaluating dose-effect experiments. J. Pharmacol and Exper. Ther., 96: 99-113.

NCR (2008) : Lost corps of Africa: Volume III, fruits, development, security and cooperation. National research council. The national Academies press, Washington D.C., USA.

Neuwinger,H.N. (2004): Plants used for poison fishing in tropical Africa Toxicon 44, 417 – 430.

Ambecher, E. (1970): Textbook of Fish diseases. T. F. H. Publications, Neptune city, New Jersy: 117-135.

Annune, P.A. and Ajike, S.E. (1999): Acute toxicity and Gill morphology of Oreochromis niloticus (Trewavas)Exposed to Rigor. J. of Aqu.sci.14: 1-4.

Austin, B. and Austin, D. A. (2007): Bacterial fish pathogens: "Diseases of farmed and wild fish", 4th revised Ed. Springer Praxis Publishing, Chichester, UK

Ayoola, S.O. (2008): Toxicity of Herbicide, Glyphosate on Nile tilapia (Oreochromis niloticus) Juvenile. Afr. J. of. Agricult.res.3(12): pp.825-834.

Ayoola,S.O.; Kuton,M.P.; Idowu,A.A. and Adelekun, A.B.(2011): Acute Toxicity of Nile Tilapia (Oreochromis niloticus) Juveniles Exposed to Aqueous and Ethanolic Extracts of Ipomoea aquatica Leaf. Nat and Sci 9(3):91

Bagalwa, M. and Chifundera, K. (2007): Environmental impact evaluation of the stem bark extract of Maesa lanceolata used in Democratic Republic of Congo Journal of Ethnopharmaco.114,281–284.

Barham, D. and Trinder, P. (1972): Enzymatic determination of uric acid. Analyzed, 97: 142-145.

Caguan, A.G.; Galaites, M.C. and Fajardo, L.J. (2004): Evaluation of botanical piscicides on Nile tilapia Oreochromis niloticus L. And mosquito fish(Gambusia affinis)Baird and Girard Proceedings on ISTA, 12-16 September. Manila, Phillipines: 179-187 .

Conroy, D.A. and Herman, L. R. (1981): Textbook of Fish diseases. T. F. H. Publication, West Sylvania

Costa, P. M.; Diniza, M. S.; Loboa, S. C.; Martinsc,J. M. Ferreirac, A. M.; Caetanoc,M. C.;Valec, T. A. And Costa , M. H.(2009): Histological biomarkers in liver and gills of juvenile Solea senegalensis exposed to contaminated estuarine sediments: Aqua. Toxicol.,92: 202–212.

Culling, C.F. (1983): Handbook of Histopathological and Histochemical techniques, 3rd ed., Butterworth, London.

Dacie, J.V. and Lewis,S.N. (1984): Practical Haematology. 6th Edition Edinburgh, Churchill Livinstone.

De-Hai,X.U. ; Julia,W. P.; Phillip,K.C. and Shoemaker,A. (2012): Parasitism by protozoan Ichthyophthirius multifiliis enhanced invasion of Aeromonas hydrophila in tissues of channel catfish. Vet.Parasitol ., 184 . 101-107.

Fafioye,O.O(2001): Lethal and sub lethal effect of extracts of Parkia biglobosa and Raphia vinifera on some freshwater fauna. J.Aqu.Sci.,16(1): 78-79

Fafioye, O.O.; Adebisi, A.A. and Fagade, S.O. (2004): Toxicity of Parkia biglosa and Raphia vinifera extracts on Clarias gariepinus juveniles. Afri. J.Biotech. 3(11): 627-630.

Francis,G.;Makkar,H.P.S.; Kerem,Z. and Becker,K. (2002): The biological action of saponins: a review. Brl. J.of Nutr.88: 587-605.

Furo ,N.A. and Ambali ,A. G. (2012): An investigation into the phytochemical constituents of Moringa oleifera aqueous bark extracts Centrepoint Journal (Science Edition, 18: pages 1 – 7.

Gupta,S.C.; Shenoy,S. and Kottech,M. (2012): Pharmacognostical and Phytochemical valuation of Balanites aegyptiaca(L.) Del. Stem bark Int.res j. pharm., 3(7):169- 172

Henry, R. J. (1974): Clinical Chemistry Principles and Techniques. 2nd ed., Harper and Row Publ., New York, USA.

Jain,N.C.(1986): Schalms veterinary heamatology 4th edition,Lea and Febiger ,Philadeelphia pp 1221.

Kensil, C.R.(1996): Saponins as vaccine adjuvants. Crit. Rev.Ther. Drug Carrier Syst. 13, 1–55.

Kew,M.L.(2000): Serum aminotransferase concentration as evidence of hepatocellula damage. Lancet 335:951-952.

Keziah, I.N. (2013) : Bioaccumulation and toxic effect of some heavy metals in freshwater fish ARI ., 10(3): 1792 – 1798.

Kostova, J. and D. Dinchev, D.(2005): Saponins in Tribulus terrestris chemistry and bioactivity. Phytochemistry Reviews, 4 (1): 111–137.

Litchfield,J.T. and Wilcoxon,F. (1949): A simplified method of evaluating dose-effect experiments. J. Pharmacol and Exper. Ther., 96: 99-113.

NCR (2008) : Lost corps of Africa: Volume III, fruits, development, security and cooperation. National research council. The national Academies press, Washington D.C., USA.

Neuwinger,H.N. (2004): Plants used for poison fishing in tropical Africa Toxicon 44, 417 – 430.

Obidah, W.; Nadro,M.S.; Tiyafo,G.O. and Wurochekke,A.U. (2009): Toxicity of Crude Balanites aegyptiaca Seed Oil in Rats. J Amer. Sci. 5, 13-16.

Okwuosa,V.N.; Molta,B.S. and Ebele,S.(1993): Toxicity of aqueous bark extract of the tree *Balanites aegyptiaca* on the fish *Oreochromis niloticus*. Appl Parasitol.34:89–94.

Omoniyi, I.; Agbon,A. and Sodunk, S.A. (2002): Effects of lethal and sublethal concentrations of tobacco (Nicotiana tobaccum), leaf dust extractum on weight and haematological changes in Clarias ganiepinus (Buchell 1822). J. Appl. Environ. Man., 6: 37-41.

Osman,H. A. M.; Ibrahim, B.T.; Soliman ,W. E. and Maather, M. M. (2010): Influence of dietary commercial Beaker's yeast, Saccharomyces cerevisae on growth performance, survival and immunostimulation of Oreochromis niloticus challenged with Aeromonas hydrophila. Nat and Sci.8(3):96-103

Reitman,S and Frankel,S.(1957): Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:53–56

Sahu, I.; Das,B.K. Marhual, N.P.J.; Pradhan,D.J. ; Behra,B. and Mishra, B. K.(2007): Phenotypic and genotypic methods for identifications of *Aeromonas hydrophila* strains from carp Labeo rohita and their virulence study. Int J Fisheries Aquacult Sci. 2(2): 141-156.

Snedecor, G. And Cochran,W.(1969): Statistical Methods 6th.ed.Iowa State Univer. Press. Atmes. Iowa, USA.

Sudhanshu,T. and Ajay,A. (2004): Piscicidal and anti-acetylcholinesterase activity of Euphorbia royleana stem bark extracts against freshwater common predatory fish Channa punctatus Environ. Toxicol.and Pharmacol.18:47–53 .

Ufodike, E.B.C. and Omoregie,E. (1994): Acute toxicity of water extracts of barks of *Balanites aegyptica* and Kigelia africana to Oreoechromis nitoticus (L). Aqua. Fish. Mgt., 25, 873-879.

الملخص العربى

تم عمل التجارب لمعرفه الآثار السامة المترتبة علي استعمال المستخلص المائي لنبات بلح الصحراء او الهجليج او اللالوب نتيجة لاستخدامه في مناطق من غرب السودان علي المسطحات المائية للصيد الغير قانوني وذلك لسهوله استخدامه, توافر هذه النباتات ولأنها تعتبر مصدر رخيص لعدم توافر معدات الصيد من شباك وغيرها لارتفاع أسعارها وأيضا لما له من تأثيرات مترتبة مستقبليه علي البيئة ومخزون الأسماك بصفه عامه وأثار صحية علي المكان المحليين بصفه خاصة. تم عمل تجارب علي 200من أسماك البلطي متوسط وزن (90-110)جرام ومتوسط طول (17-19)سم بهدف تحديد التالي:-

- التركيز النصف مميت وذلك خلال 96 ساعة علي اسماك البلطي والتي كانت 69ملجم/ لتر. ووجد ان هناك علاقة بين سميه المستخلص المائي للأسماك مع التركيز ومده التعرض للتركيز السام.
- دراسة تأثير تعرض اسماك البلطي للمستخلص المائي لنبات الهجليج عند تركيز ½ من التركيز النصف مميت خلال 96 ساعة وملاحظه الأعراض الاكلينيكه
- دراسة تأثير تعرض اسماك البلطي للمستخلص المائي لنبات الهجليج عند تركيز 10/1 من التركيز النصف مميت خلال 96 ساعة وملاحظه الأعراض الاكلينيكه .
- بأجراء الفحص الدموي علي عينات من الدم الخاص من اسماك البلطي والتي تعرضت الي للمستخلص المائي لنبات الهجليج عند تركيز½ وتركيز 10/1من التركيز النصف مميت خلال 96 ساعه وجد ان الفحص قد صوحب بازدياد شديد في كريات الدم الحمراء وفي نسبه تركيز الهيموغلوبين وحجم الترسيب التراكمي خاصة في حاله التعرض المزمن للأسماك لمده 8 أسابيع متتالية. بينما لوحظ ازدياد ملحوظ في عدد كريات الدم البيضاء بعد التعرض الحاد لمده 7 ايام في اسماك البلطي .
- تم رصد العلامات الاكليتيكه لأسماك البلطي التي تعرضت للمستخلص المائي لنبات الهجليج عند تركيز ½ من التركيز النصف مميت خلال 96 ساعه وقد لوحظ ظهور انماط غير طبيعيه وتغير في السلوك عند السباحه للاسماك من هروب واختباء الأسماك بالاضافه الي فقدان الاتزان والسباحه الجانبيه حتي الموت, اضطرابات تنفسيه مصحوبه باحتقان حاد في الخياشيم مع وجود مخاط كثيف في الخياشيم و الجلد, ونزيف وتاكل في الزعانف.
- كما اظهرت ألصفه التشريحية احتقان بالاضافه الي تضخم الكبد عند التعرض ل ½ من التركيز النصف مميت خلال 96 ساعه وقد لوحظ صحة و نشاط الأسماك مع عدم ظهور أعراض اكلينيكه ما عدا اضطرا بات تنفسيه في بعض الحالات القليله
- تم رصد العلامات الاكليتيكه لأسماك البلطي التي تعرضت ل تركيز 10/1 من التركيز النصف مميت خلال 96 ساعه وقد ولوحظت
- أظهرت ألصفه التشريحية احتقان وتضخم في الكبد في بعض الحالات القليله
- تم عمل دراسه هستوباثولوجيه علي اسماك البلطي عند تعرضها لتركيزات مختلفة من المستخلص المائي لنبات الهجليج ولمدد مختلفه وقد ولوحظت نغيرات هستوباثولوجيه في الخياشيم في صوره زياده في إعداد الاشعه الخيشوميه وارتشاحات مائية مع التصاق في الخياشيم ولوحظ في الكبد احتقانات في الاوعيه الدمويه للكبد مع وجود فجوات في خلايا الكبديه في حاله التعرض ل ½ من التركيز النصف مميت خلال 96 ساعه في بعض الحالات القليله
- نغيرات هستوباثولوجيه في الكبد في حاله التعرض ل 10/1 من التركيز النصف مميت خلال 96 ساعه وجد ان
- تم عمل دراسة عن تأثير التعرض للمستخلص المائي لنبات الهجليج عند تركيز ½ وتركيز 10/1 من التركيز النصف مميت خلال 96 ساعه وجد ان الفحص قد صوحب بازدياد شديد في معدلات الإنزيمات الخاصة بالكبد بينما لا يوجد أي تغيير في معدلات اليوريا والكرياتنين.
- تم عمل تحليل لمستبقيات المستحضر المائي لنبات الهجليج في انسجه عضلات اسماك البلطي بعد مرور 8 أسابيع من التعرض وتم اكتشاف وجود بقايا للمستخلص المائي لنبات الهجليج في انسجه عضلات الأسماك مقارنه بالمجموعة الضابطه.
- كان تعرض اسماك البلطي للمستحضر المائي لنبات الهجليج عند تركيز10% من التركيز النصف مميت تاثير ايجابي علي صحة الأسماك حيث لوحظ انخفاض معدلات النفوق في بصوره ملحوظة بعد الحقن التجريبي لبكتريا *A.hydrophila* من خلال تقييم مقارن بالمجموعة الضابطة.
- من هذه النتائج يمكن استخلاص ان الصيد الغير قانوني بواسطه المستخلص المائي لنبات الهجليج له تأثير ضار جدا ويعتبر كارثه عند استعماله بكميات وتراكيز عاليه لما له تاثير سلبي علي مجموعات الأسماك والبيئة مسببا فقد اقتصادي نتيجة لنفوق إعداد كبيرة من الأسماك بالاضافه للتأثير المباشر علي انتاجيه وتكاثر الأسماك