African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 3, Issue 3,July-September2024, Page No: 213-219
Website: *https://aaasjournals.com/index.php/ajapas/index*
معامل التأثير العربي 2023: (1.55) SJIFactor 2023: 5.689 ISI 2022-2023: 0.557

Effect of Laundry Soap Containing Linear Alkyl Benzene Sulfonate on Protein and Cellular Metabolic Activity of Gills and Liver Primary Cell Culture of Shield head Fish (*Synodontis schall*)

Muzn Tarig Khalid Abdurahim [1*], Shima Elfadel Ahmed Hassan [2]
[1] Dept. of Biology and Biotechnology, Faculty of Science and Technology, University of Al-Neelain, Khartoum, Sudan
[2] Ministry of Higher Education and Scientific Researches, National Center for Research, Khartoum, Sudan

تأثير صابون الغسيل المحتوي على سلفونات ألكيل البنزين الخطي على البروتين والنشاط الايضي الخلوية لخلايا الخياشيم والكبد الاولية المستزرعة لأسماك القرقور (سينودونتيس شال)

مزن طارق خالد عبد الرحيم[1*]، شيماء الفاضل احمد حسن[2]
[1] قسم الاحياء والتقنيات الإحيائية، كلية العلوم والتقانة، جامعة النيلين، الخرطوم، السودان
[2] وزارة التعليم العالي والبحث العلمي، المركز القومي للبحوث، الخرطوم، السودان

[*]Corresponding author: mazoonkhalid25@gmail.com

Received: July 14, 2024	Accepted: August 31, 2024	Published: September 09, 2024

Abstract:
One of the main sources of chemical water pollutants for fish is the laundry soap that we use in our daily lives without adequate treatment to remove harmful compounds. The present study aimed to develop cost-effective bioassays to study the effects of laundry soap containing linear alkyl benzene sulfonate on Shield Head Fish (Synodontis Schall) gills and liver primary cell culture. The primary cell culture was carried out following a method modified by Gaurav *et al.* [1] It was cultured in petri dishes containing omega-3 medium. The bioassays were carried out when the culture reached up to 100% confluence in 24 days by using four different concentrations of laundry soap (10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml) for 48 hours to observe changes in protein biomass and cellular metabolic activity. The results showed no significant difference in protein concentration between doses. These changes were in direct correlation with the dose value, but cell viability decreased with increased doses on gills and liver primary cells as determined by reducing the MTT reagent to formazan indicating a potential concern for the health of fish and aquatic invertebrates. The study suggests using Omega-3 as a growth medium for primary cell cultures and candle smoke to promote cell growth and as a source of carbon dioxide. There must be more research on purifying water and separating harmful substances from it and efforts must be made to create environmentally friendly soap from natural materials.

Keywords :laundry soap, linear alkyl benzene sulfonate, Cellular Metabolic Activity, Gills and Liver Primary Cell Culture, Shield head fish (*Synodontis Schall*) .

الملخص:
أحد المصادر الرئيسية لملوثات المياه الكيميائية للأسماك هو صابون الغسيل اليومي الذي نستخدمه في حياتنا اليومية دون معالجة كافية لإزالة المركبات الضارة. كان الهدف من هذه الدراسة هو تطوير اختبارات حيوية فعالة من حيث التكلفة لدراسة آثار صابون الغسيل الذي يحتوي على سلفونات ألكيل بنزين الخطية على خياشيم سمكة الدرع (سينودونتيس شال) وثقافة الخلايا الأولية للكبد. تم إجراء زراعة الخلايا الأولية باتباع الطريقة التي تم تعديلها بواسطة لعالم قوارف واخرون [١] تمت زراعتها في أطباق بتري تحتوي على وسط أوميغا 3. تم إجراء الاختبارات الحيوية عندما وصلت الثقافة إلى التقاء 100٪ في 24 يومًا باستخدام أربعة تراكيز مختلفة من صابون الغسيل (10 مجم / مل، 5 مجم / مل، 2.5 مجم / مل، و 1.25 مجم / مل) لمدة 48 ساعة لمراقبة التغيرات في الكتلة الحيوية البروتينية والنشاط الأيضي الخلوي. وأظهرت النتائج عدم وجود اختلاف كبير في تركيز البروتين بين الجرعات. كانت هذه التغييرات مرتبطة بشكل مباشر بقيمة الجرعة لكن صلاحية الخلية انخفضت مع زيادة

الجرعات على الخياشيم وخلايا الكبد الأولية، كما هو محدد عن طريق تقليل كاشف MTT إلى فورمازان، مما يشير إلى قلق محتمل على صحة الأسماك واللافقاريات المائية. تقترح الدراسة استخدام أوميغا 3 كوسيلة نمو لمزارع الخلايا الأولية ودخان الشموع لتعزيز نمو الخلايا وكمصدر لثاني أكسيد الكربون. ويجب إجراء المزيد من الأبحاث حول تنقية المياه وفصل المواد الضارة عنها، ويجب بذل الجهود لصناعة صابون صديق للبيئة من مواد طبيعية.
الكلمات المفتاحية: صابون الغسيل، سلفونات ألكيل البنزين الخطي، النشاط الايضي الخلوي، خلايا الخياشيم والكبد الاولية المستزرعة، أسماك القرقور (سينودونتيس شال).

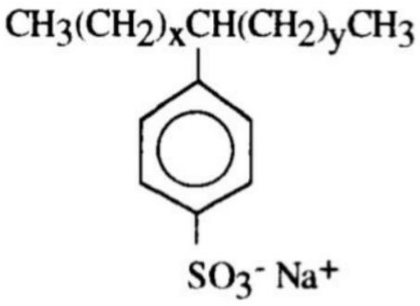
Introduction

Fish cell cultures have gained prominence due to advancements in research and ethical pressure to reduce animal testing. Animal activist organizations, environmental organizations, and the cosmetics industry promote alternative in-vitro models, offering benefits such as simple medication dosing, repeatability, quick test findings and economic viability [2].

Detergents are cleaning products made from synthetic organic compounds, with surfactants being the main ingredients. Commercial detergents typically contain 10%–20% surfactants and other ingredients like bleach, filler, foam stabilizer, builders, fragrances, enzymes, dyes, and optical brighteners. The most popular surfactant is linear alkyl benzene sulfonate (LAS)-ionic surfactant, which degrades more quickly than ABS in the sea. Inadequate degradation can cause harm to aquatic life and cause hematological, hormonal, and enzyme abnormalities [3].

Linear alkyl benzene sulfonate (LAS) is the primary anionic surfactant in laundry detergents, with its usage increasing from 600 to 850 million pounds in the US over the past decade. LAS is effectively removed from wastewater and the environment through biodegradation, sorption/settling, and wastewater treatment techniques. However, data gaps exist in trickling filters, oxidation ditches, RBCs, and lagoon treatment facilities [4].

The family Mochokidae consists of three genera: Synodontis, Chiloglanis, and Mochokus, of which the genus Synodontis is the most common and of great commercial importance. Synodontis species only occur in Africa and a portion of those species present in the Nile are restricted to water systems within the tropics [5].



Linear Alkyl Benzene Sulfonate (LAS)

Material and Methods

1. Establishment of Gills and Liver Primary Cell Culture:

Materials:

Glass aquaria, Omega-3, Tissue culture Petridishes, Scissors, Phosphate buffer solution (PBS), EDTA, 20% streptomycin and penicillin, foil, Candle and, Refrigerator.

Method:

The cell culture was established using the scientific method modified by Gaurav et al. Gills and liver tissues were cut into small fragments of 1.0 mm, washed twice with phosphate buffer solution (PBS) and transferred to a Petri dish containing 20% streptomycin and penicillin for 5 minutes. The cells were dissociated using 0.2 g of EDTA dissolved in 10 ml of phosphate buffer solution (PBS) for 2 minutes, and then the cells were centrifuged at 2000 rpm for 1 minute. Finally, the pellets were seeded in Petri dishes containing omega-3 medium and given CO2 by burning out a candle. The Petri dishes were covered with foil and kept in the refrigerator at 4 °C. The culture medium was changed every 6 days for 24 days and counted every 7 days for 28 days. After 24 days, the primary cell culture formed a full monolayer and reached 100% confluence.

2. Total Cell Protein Assay:

Materials:
Phosphate Buffer Solution, 0.1 mm NaOH, Coomassie Blue solution and Spectrophotometer.

Method:
The cell protein assay adopted by Shopsis and Eng [6] to measure the total cellular protein after incubation in descending concentrations of medium containing linear alkyl benzene sulfonate (LAS) (10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml) and tubes containing only omega-3 medium were kept as controls. After 48 hours of incubation, the medium was removed, the cells were washed with PBS and 1 ml of NaOH was added to each tube. The plate was incubated for 1 hour at 4 °C then 1 ml of Coomassie Blue solution was added to each tube and incubated at room temperature for 20 min. The absorption of each tube was measured at 595 nm. Serial dilutions of 1 to 100 mg/mL BSA dissolved in 0.1 mL NaOH were used for the protein standard. All experiments were performed three times and the average absorbance at each dose was calculated. The value of protein was determined according to the Bovine Serum Albumin (BSA) standard curve ($y = 0.0167x + 0.0561$).

3. Mitochondrial Suicidical Dehydrogenate Assay (MTT):

Materials:
Tetrazolium Dye MTT, Dimethyl Sulfoxide and Spectrophotometer

Method:
The method was done according to Borenfreund et al. [7], which was based on the reduction of the soluble yellow tetrazolium salt (MTT) to a blue insoluble MTT formazan. The reduction done by the suicidal dehydrogenate that was produced from the mitochondria that had been injured by descending concentrations of soap containing linear alkyl benzene sulfonate (LAS) (10 mg/ml, 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml) and tubes containing only omega-3 medium were kept as controls. After a 48-hour exposure period the tested medium was removed and 20 mL of 5.0 mg/mL MTT was added to phosphate buffer solution (PBS) and incubated for 4 hours at 4 °C. The cells were washed twice with PBS and 1 ml of dimethyl sulfoxide was added to each tube to dissolve the purple formazan crystals that had been produced. The absorbance of each tube was measured at 490 nm. The experiments were performed three times and the average absorbance at each dose was calculated and expressed as a percentage.

3.Statistical Analyses:
Data were subjected to analysis of variance (ANOVA) using SPSS to compare the results and linear correlation between variables was also carried out using the Pearson correlation coefficient.

Results:
1. Establishment of Gills and Liver Primary Cell Culture:
Primary cell cultures have been established from tissues and have shown an increase in the number of live cells every 7 days. The viability of gills and liver primary cells showed that omega-3 was a suitable medium for growing and maintaining the viability of gill and liver primary cell cultures (Figures 1, 2 and 3)

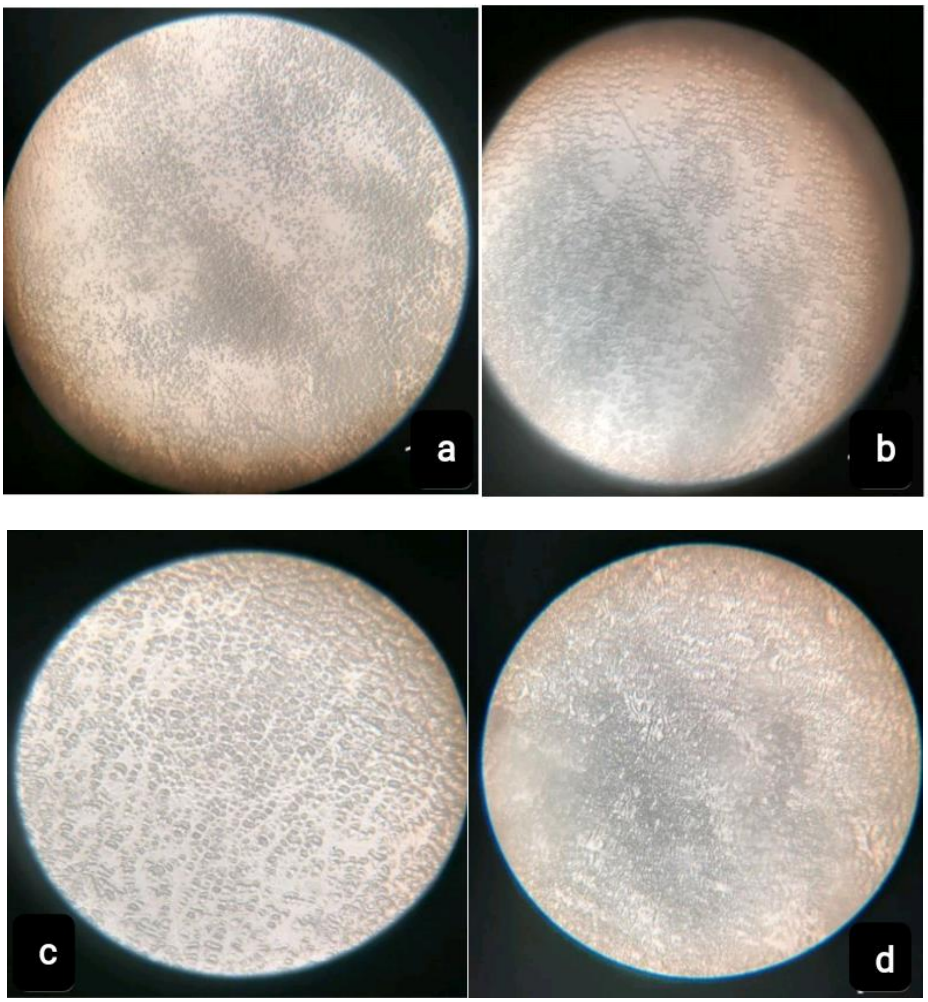
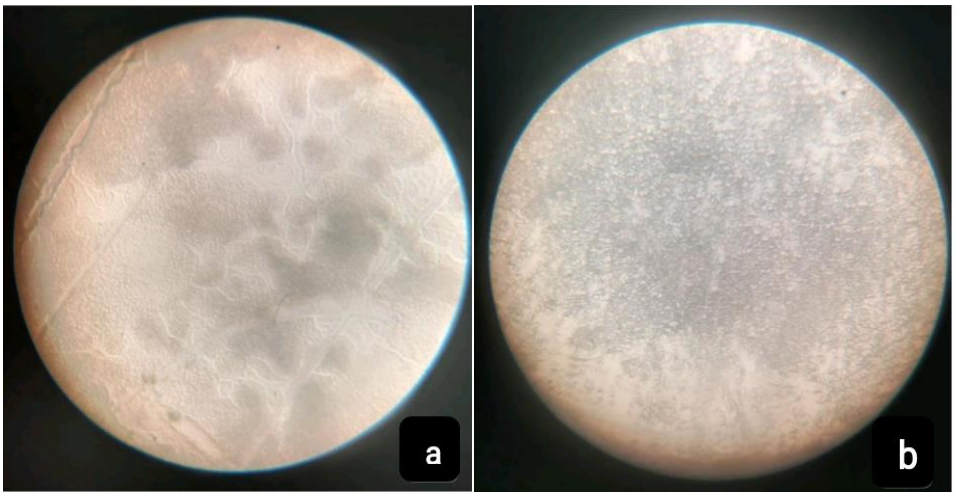


Figure 1 Gills primary cell culture grown in omega-3 medium in (a) 6 days (b) 12 days (c) 18 days (d) 24 days (Eosinx100).



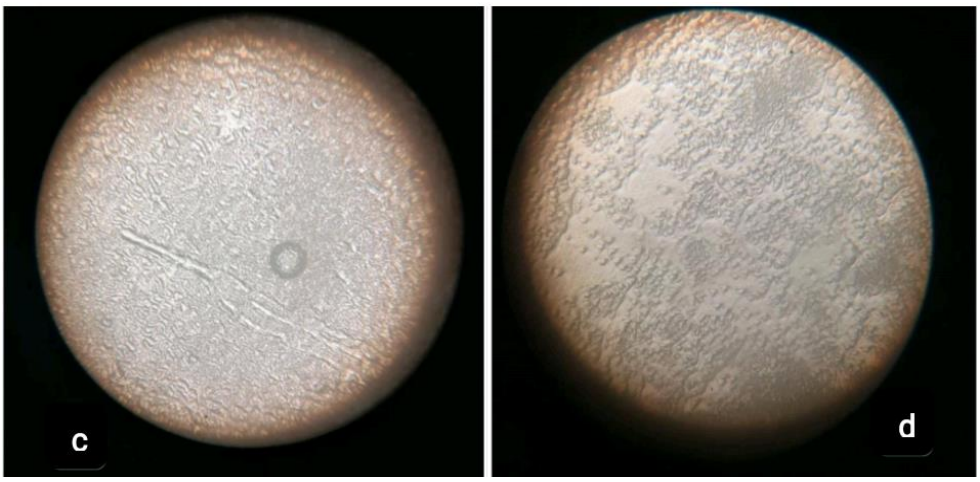


Figure 2 Liver primary cell culture grown in omega-3 medium in (a) 6 days (b) 12 days (c) 18 days (d) 24 days (Eosinx100).

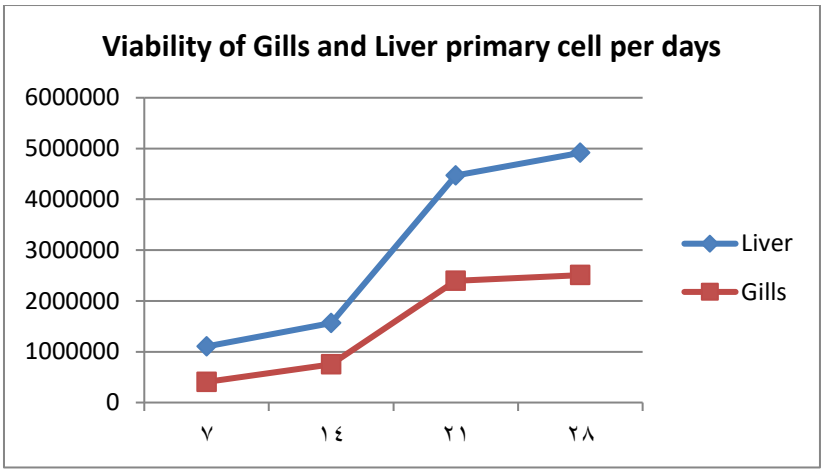


Figure 3 Viability of gills and liver primary cell in omega 3 medium per day: 7 days,14 days, 21 days and 28 days.

2. In-vitro Total Prote in Bioassay:

The total protein of gills and liver primary cells was affected by doses after 48 hours of exposure. The value of protein was determined according to the Bovine Serum Albumin (BSA) standard curve as shown in table 1.

Table (1). Effect of four different concentrations of laundry soap containing linear alkyl benzene sulfonate (LAS) on Shield head (*Synodontis schall*) gills and liver primary cells on protein amount after 48 hours exposure.

Dose in mg/ml	Type of cells	Value of cell protein	Concentration of cell protein	significant
0.00	Gill	19.38	100	0.201
1.25		1.41	7.27	
2.5		0.21	1.08	
5		0.005	0.02	
10		0	0	

0.00	Liver	6.88	100	
1.25		4.37	68.52	
2.5		3.59	52.18	
5		2.87	41.71	
10		1.01	14.68	

3. Mitochondrial Suicidal Dehydrogenase Assay (MTT):

This colorimetric assay used the reduction of tetrazolium salt as a means to measure cellular metabolic activity for cell viability. Viable cells contained the NADpH-dependent oxidoreductase enzyme which reduced the MTT reagent to formazan and the deep purple colour was formed. The effect is also expressed by the degree of cell viability, as shown in table 2 which shows a reduction of viable cells and thus a reduction of formazan.

Table (2). Effect of four different concentrations of laundry soap on Shield head (*Synodontis schall*) gill and liver primary cells on cell viability after 48 hours treatment.

Dose mg/ml	Type of cells	% Viable cells	significant
0.00	Gill	100%	0.00
1.25		14.24	
2.5		7.97	
5		6.83	
10		1.70	
0.00	Liver	100%	
1.25		24.19	
2.5		17.47	
5		10.12	
10		8.87	

Discussion:

The present study showed that omega-3 is a suitable medium for maintaining the viability of gill and liver primary cell cultures for more than 30 days. The biochemical test showed a decrease in total protein concentrations with doses. The results agreed with Nkpondion *et al.* [8] who studied the toxicities of commercial detergent (Ariel ™; linear alkyl benzene sulfonate) a household cleaning agent on some enzymatic and protein activities of juvenile African mud catfish (Clarias gariepinus). The median lethal concentrations (LC50) derived used the Finney probit method while protein and enzymatic activities were determined using the Biuret and Randox methods respectively. Gouda *et al.* [9] who investigated the influence of linear alkyl benzene sulfonate (LAS) on the biochemical parameters of Nile Tilapia (Orechromis niloticus) total proteins showed a significant decrease at the lethal concentration (LC50) for 96 h of Ariel ™ at a concentration of 10 mg/L. Pechiammal and Vasanthi [10] found that the amount of protein, carbohydrate and lipid content in Labeorohita exposed to detergent (Tide) decreased in all experimental groups when compared to the control. The consequences of laundry soap containing linear alkyl benzene sulfonate (LAS) on the gill and liver primary cells of Shield head fish were also observed in the oxidative stress of mitochondrial activity. Viable cells contained the NADpH-dependent toxidoreductase enzyme which reduced the MTT reagent to formazan. A deep purple colour was formed and the degree of cell viability was reduced thus reducing formazan. The results agreed with Priya *et al.* [11] who studied the efficacy of synthetic detergents on hepatocyte changes in Tilapia mossambica the results suggested the detergents affected the fish morphology and physiological processes and investigated the biochemical and metabolic aspects of T. mossambica fish. Victor *et al.* [12] examined the morphological

changes in the gill of Tilapia zilli after being exposed to two sublethal concentrations (0.50 and 1.00 mg/L) of alkyl benzene sulphonate under laboratory conditions these changes in the gills resulted in erosion of the epithelial cells, fusion of filaments and extensive disruption of the epithelial membrane at higher LAS concentrations. *In-vitro* studies by Varsha *et al*. [13] determine the effect of linear alkyl benzene sulfonate on the liver tissues of Puntius ticto fish. The results showed the toxicity of LABS in the liver of Puntius ticto fish by histopathological study the liver tissues revealed cytoplasmic vacuolar degeneration, nuclear pyknosis, necrosis and blood cell infiltration as the common lesions in the hepatocytes.

Conclusion:

Total damage to the gills and liver cells of the *Synodontis schll* treated by even low doses of laundry soap containing linear alkyl benzene sulfonate (LAS) was demonstrated. The results indicated that laundry soap containing linear alkyl benzene sulfonate (LAS) induced cytotoxicity via biochemical disturbances and cell molecular injuries in the different types of cells of Shield head fish (*Synodontis schall*) in different doses through time response. However, the use of industrial laundry soap that contains the substance under study which causes water pollution and toxicity for aquatic organisms must be properly disposed of and adequate treatment must be provided to remove harmful compounds before discharge into water bodies. And by comparing laundry soaps containing linear alkyl benzene sulfonate (LAS) with regular soaps they are more widely spread and used for their effectiveness in removing dirt quickly.

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