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Semi quantitative analysis of IGF-1 and CYP1A genes expression of cultured *Mugil capito* with different sizes from different regions at Kafr El Shiekh governorate, Egypt

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ABSTRACT

The aim of this study was to evaluate the relationship between fish size and expression of insulin like growth factor-1(*IGF-1*) in musculature and cytochrome P450 1A (*CYP1A*) in liver tissues using semi-quantitative PCR. *Mugil capito* with different sizes obtained from three different regions (Al-Hamol, Al-Riad and Sidi Salem) at Kafr El Sheikh Governorate, Egypt were used. The results of *IGF-1* and *CYP1A* genes expression analysis revealed that small sized fish at Al-Hamol and Al-Riad regions were higher than those obtained from Sidi Salem region. Meanwhile, *IGF-1* expression in large sized fish at Sidi Salem region was strongly significant compared to all different sized fish in different regions. However, the *CYP1A* expression in medium sized fish at Sidi Salem region was strongly significant compared to all different sized fish in different regions. It can be concluded that pesticides pollution had effect on the fish health, which was reflected on the size and expression of *IGF-1* and *CYP1A* genes.

Keywords: *Mugil capito*, *IGF-1*, *CYP1A*, semi-quantitative gene expression.

INTRODUCTION

Mullet cultivation is a vital contributing element. The development and growth of cultivation sector in Egypt wasn't doable without mullet as a vital cash crop. All the same, mullet are refined typically in extensive and semi-intensive pond systems that expanded during a large number of countries worldwide. Currently, Egypt could be a leading country in mullet cultivation and its activities rely on the utilization of wild seed, e.g. Egypt (**Suloma and Ogata, 2006**)

Insulin like growth factor-1(IGF-1) plays a very important role in regulation differentiation, development, growth and reproduction in fish. Surprisingly, fish muscle has a larger abundance of IGF-1R subsequently IGF-1 contributes more to control muscle function (**Parrizas et al., 1995**). Many studies indicated the effect of pesticides on expression of IGF-1 in fish; of those observed a significant decrease in mRNA expression levels of IGF-I in muscles of rainbow trout during deltamethrin exposure **(Aksakala *et al.*, 2010)**, and in male tilapia during dichlorodiphenyldichloroethylene (DDE) and heptachlor exposure**(Davis *et al.*, 2009)** which might cause undesirable outcomes in growth, development and reproduction.

The fish enzyme cytochrome P4501A (*CYP1A*) carries out oxidation reactions relevant to xenobiotic biotransformation. Thus, induction of *CYP1A* can play an important role for assessing environmental contamination **(Bucheli and Fent, 1995)**. Apparently, the hepatic level CYP1A mRNA was induced in response to pollutants **(George e*t al.*, 2004)**. Therefore, these changes are very important tool for diagnosis of chemical pollution and pesticides in fish. The aim of this study was to assess the impact of pesticides residues on the *IGF-1*and *CYP1A*gene expression.

MATERIAL AND METHODS

Fish sampling

Total number of 150 *Mugil capito* were collected from the study regions (Al-Hamol, Al Riad and Sidi Salem) fish farms. Fish were divided into three groups based on body weight; less than 200 (small, S), between 200-300g (medium, M) and more than 300g (large, L). Liver and dorsal musculature were excised, packed and kept at -80^{o}C.

RNA extraction

TRIzol method were used for RNA extraction from liver and musculature according to manufacturer's instructions and following **(Chomczynski, 1993).** The quantity of RNA was evaluated by using Nano-

drop spectrophotometer and purity by OD_{260}/OD_{280} nm absorption ratio 1.8:2.0.

PCR reaction and program

The isolated cDNA were amplified using script RT-PCR two-step kit following the manufacturer protocol (Jena Bioscience, Germany). The used primers obtained using primer3 tool http://primer3.ut.ee/cgi-bin/primer3/. PCR amplification was carried out by SensoQuest (Labcycler, Germany) using a 50 µl of polymerase chain reaction mixture contained: 2µl of cDNAused as template separately, 5 µl of 10x Hot Start Buffer complete, 1µl of dNTP Mix, 0.25µl of Hot Start Pol, 1µl of forward primer (0.1-0.5 µM), 1µl of reverse primer (0.1-0.5 µM) and 39.75µl of RNase free Water. The final reaction mixture was placed in a thermal cycler and the PCR program was carried out by initial denaturation at 94 ^{o}C for 2 min followed by 40 cycles of 94 ^{o}C for 30 sec for DNA denaturation, annealing temperatures as seen in (**Table 1**) for 30 sec, extension at 72 ^{o}C for 1 min and final extension at 72 ^{o}C for 10 min then were held at 4 ^{o}C. Amplified PCR product was analyzed by electrophoresis in 2 % agarose gel stained with Ethidium bromide using 50 bpDNA ladder (Thermo Scientific, USA), then visualized under UV Trans-illuminator.

Table 1: The used primers sequences in the current study.

Gene of interest	Primer sequences (5' - 3')	Ta (^{0}C)	Amplification size (bp)	Reference
β- actin	**F**:CCACGAGACCACCTACAACA **R**:CTCTGGTGGGGCAATGAT	51.2	181	(Bangcaya, 2004)
IGF-1	**F**:CTGTAGCCACACCCTCTCAC **R**:CAGTACATTTCCAGGCGCC	53.3	240	(GenBank:AY427954)
CYP1A	**F:**CCTGTCGTGGTCAGTGATGT **R**:TTTGTGGTGCAGTGTGGAAT	50.9	161	(GenBank:AY827103.1)

Statistical analysis

Quantification of band intensities (OD) was measured using Image J software and a ratio OD candidate genes compared to OD of β-actin were calculated for each gene. Probability associated with one way ANOVA.

RESULTS

IGF-I mRNA levels

The expression levels of *IGF-I* gene of *Mugil capito* in dorsal musculature was significantly increased compared to *β-actin* gene at Al-Hamol and Al-Riad regions at both small and medium sized fish, while the large size showed the lowest expression (**Fig 1**and**2**).While mRNA expression ofthe large size (L) at Sidi Salem region showed the highest expression compared to medium and small sized fish (**Fig 3**).

CYP1A mRNA level in liver

It was noticed from the obtained results that mRNA expression of *CYP1A* gene of small size (S) and medium size (M) *Mugil capito* showed the highest expression at Al-Hamol and Al Riad regions, but the large size (L) showed the lowest expression (**Fig 4,5)**. On the other hand, at Sidi Salem region; mRNA expression of *CYP1A* gene of large andmedium sized fish showed the highest expression, but the small size showed the lowest expression (**Fig 6)**.

DISCUSSION

The present study is an extension to a work **for the same authors** (under publishing) where the authors did survey on pesticide residue in the studied areas (Al Hamol, Al Riad and Sidi-Salem) for the fish farm water and tissues of cultured *Mugil capito*. But this study focuses on the impact of pesticides pollution on IGF and CYP1genes expression in relation to the fish size at each study area.

Insulin like growth factor-1(IGF-1) plays an important role in regulating differentiation, development, growth and reproduction in fish. Surprisingly, fish muscle has a greater abundance of IGF-1R subsequently IGF-1 contributes more to regulate muscle function (**Parrizas *et al.*, 1995**). And it was noticed that fish exposed to pesticide pollution exhibited inhibition in growth; leading necessity for evaluation of IGF activity in response to xenobiotic chemicals **(Denslow *et al.*, 2002)**. On the level of the same areas, significant induction in IGF-1expression in dorsal musculature in small sized *Mugil capito* at Al Hamol and Al Riad regions were noticed while large sized fish showed decrease in IGF-1 expression. It was suggested that expression of IGF-1 gene in dorsal musculature in these regions is likely to be influenced by accumulated pesticides residues in tissues. The expression of IGF-1was induced in small size than large size that it was might be no significant accumulation of pesticides residues in their tissues. Previous study examined Tilapia exposed to o, p–DDE and heptachlor for long period and found suppression in IGF-I expression levels **(Davis *et al.*, 2009)**.On the other hand, a significant induction of IGF-1 expression in dorsal musculature of large sized *Mugil capito* at Sidi salem region and suppressed its expression in small sized fish was reported in this study. This may be attributed to amount of pesticides residues in muscles of large sized fish was more than in small size. Similarly, exposure to high level of pesticides for short term showed increase IGF-1 expression **(Davis *et al.*, 2009**). Because the pesticides may have exerted their effects by stimulation of thyroid hormone release that have been shown to stimulate hepatic IGF-1 expression **(Schmid *et al.*, 2003)**. Therefore, the growth

reducing effect was associated with significant changes in hepatic IGF-1 expression.

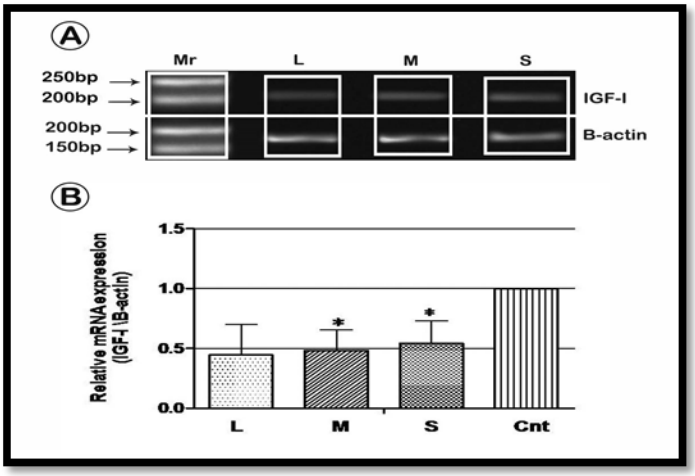

Fig 1: Differential analysis of ***IGF-I*** expression gene levels in dorsal musculature of *Mugil capito* collected from Al-Hamol. **A)** Ethidium bromide stained agarose gel of purified clone of ***IGF-I*** gene with size of 240bp (upper gel) compared to the housekeeping gene (***β-actin***) with size 181bp (lower gel) and **Mr** represented by ladder 50bp. **B)** Band intensity was quantified using Image J software and the ratio of OD ***IGF-I*** gene /OD ***β-actin*** was calculated after PCR.

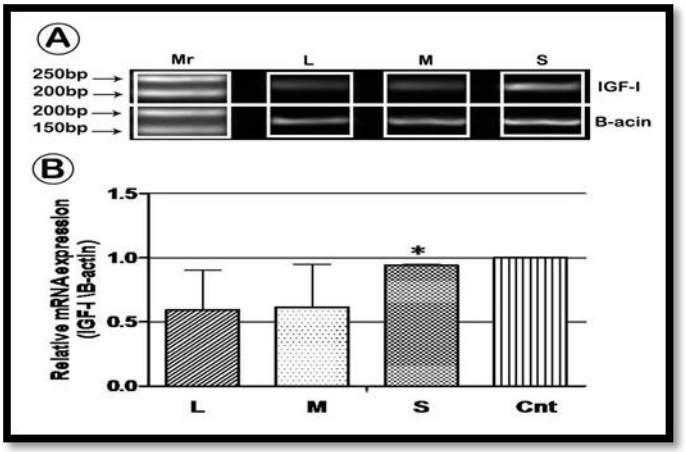

Fig 2: Differential analysis of ***IGF-I*** expression gene levels in dorsal musculature of *Mugil capito* collected from Al-Riad. **A)** Ethidium bromide stained agarose gel of purified clone of ***IGF-I*** gene with size of 240bp (upper gel) compared to the house keeping gene (***β-actin***) with size 181bp (lower gel) and Mr represented by 50bpladder. **B)** Band intensity was quantified using Image J software and the ratio of OD ***IGF-I*** gene /OD ***β-actin*** was calculated after PCR.

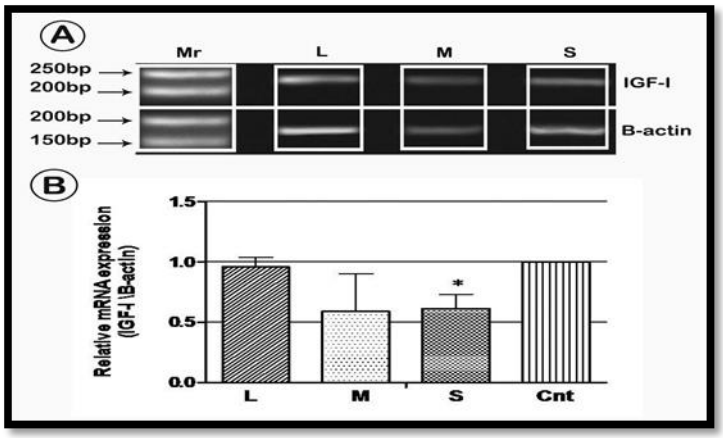

Fig 3: Differential analysis of ***IGF-I*** expression gene levels in dorsal musculature of *Mugil capito* collected from Sidi salem region. **A)** Ethidium bromide stained agarose gel of purified clone of ***IGF-I*** gene with size of 240bp (upper gel) compared to the house keeping gene (***β-actin***) with size 181bp (lower gel) and **Mr** represented by ladder 50bp. **B)** Band intensity was quantified using Image J software and the ratio of OD ***IGF-I*** gene /OD ***β-actin*** was calculated after PCR.

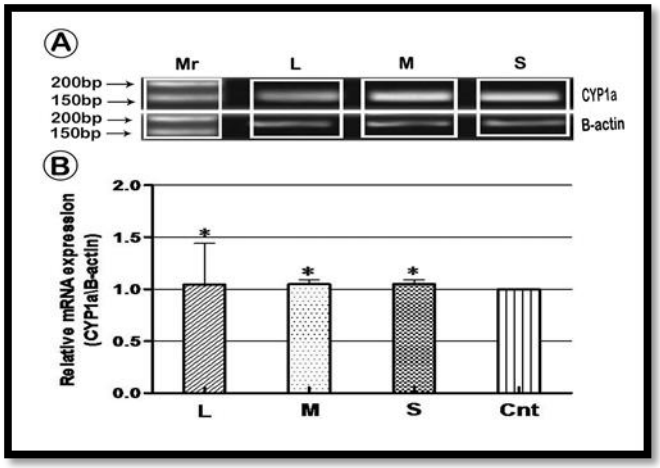

Fig 4: Differential analysis of ***CYP1A*** expression gene levels in liver of *Mugil capito* collected from Al-Hamol region. **A)** Ethidium bromide stained agarose gel of purified clone of ***CYP1A*** gene with size of 161bp (upper gel) compared to the house keeping gene (***β-actin***) with size 181bp (lower gel) and **Mr** represented by ladder 50bp. **B)** Band intensity was quantified using Image J software and the ratio of OD ***CYP1A*** gene / OD ***β-actin*** was calculated after PCR.

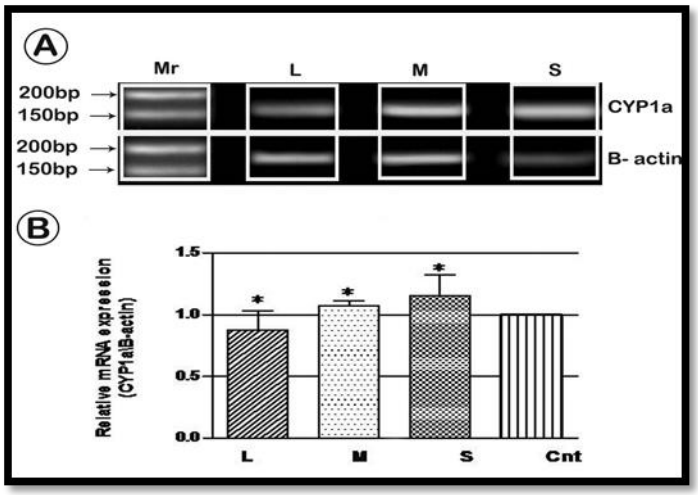

Fig 5: Differential analysis of ***CYP1A*** expression gene levels in liver of *Mugil capito* collected from Al-Riad region. **A)** Ethidium bromide stained agarose gel of purified clone of ***CYP1A*** gene with size of 161bp (upper gel) compared to the house keeping gene (***β-actin***) with size 181bp (lower gel) and **Mr** represented by ladder 50bp. **B)** Band intensity was quantified using Image J software and the ratio of OD ***CYP1A*** gene /OD ***β-actin*** was calculated after PCR.

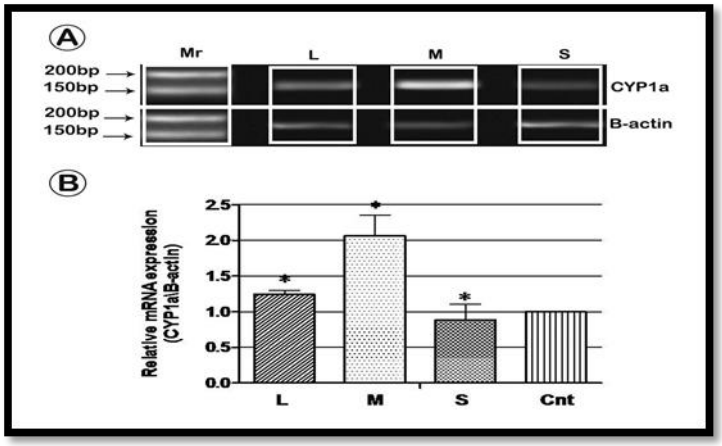

Fig 6: Differential analysis of ***CYP1A*** expression gene levels in liver of *Mugil capito* collected from Sidi salem region **A)** Ethidium bromide stained agarose gel of purified clone of ***CYP1A*** gene with size of 161bp (upper gel) compared to the house keeping gene (***β-actin***) with size 181bp (lower gel) and **Mr** represented by ladder 50bp. **B)** Band intensity was quantified using Image J software and the ratio of OD ***CYP1A*** gene /OD ***β-actin*** was calculated after PCR

CYP1A gene expression was significantly higher in both small and medium sized fish compared to large size from the same ponds and same areas nearly in all study regions. The results of **(Jönsson *et al.*, 2007; Kim *et al.*, 2008)** was in agreement with our results that showed higher induction of CYP1A gene expression in fish exposed to some xenobiotics. In addition, another study of**(Bucheli and Fent, 1995)** demonstrated that CYP1As enzymes in liver are strongly induced by some organic contaminants. On contrast, there was no significant impression in CYP1A levels in fish exposed to different pesticides (**Wheelock *et al.*, 2005**). There are some possible explanations that in natural environment may be either a high inter-individual variation or due to simultaneously action between inducers and inhibitors of CYP1A system **(Cajaraville *et al.*, 2000; Hartl *et al.*, 2007)**. It is worthy said that, in the present study there was variations in CYP1A expression among the different sizes in all study regions. It may be also due to different concentrations of pesticides in water of different study regions. In addition, rapid biotransformation of pesticides in liver lowered the concentration in tissues of large sized fish. Previous studies indicated a significantly induction in CYP1A gene expression in tissue specific in early stages of fish after exposure to organic contaminants **(Hanno *et al.*, 2010; Woo *et al.*, 2009**). Other studies observed induction of CYP1A gene in liver of zebra fish **(Xing *et al.*, 2014)** and tilapia **(Hassanin *et al.*, 2009; Neilson, 2000)**when exposed to pesticides.

It could be concluded that, IGF1 and CYP1A genes can be used as sensitive biomarker for assessment contamination in all stages of fish.

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التعبير الجيني لجيني الانسولين عامل النمو -١ و السيتوكروم ١أ في اسماك البوري في أماكن مختلفه في محافظه كفرالشيخ- مصر

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الملخص العربي

هدف الدراسه هو تقيم تاثير التلوث بالمبيدات الحشريه علي التعبير الجيني لجين الانسولين عامل النمو -١ في انسجه العضلات و جين السيتوكروم١أ في انسجه الكبد باستخدام تفاعل البلمرة المتسلسل في اسماك البوري خلال ثلاث أماكن مختلفه في محافظه كفر الشيخ , مصر . بينت النتائج ان التعبير الجيني لجين الانسولين عامل النمو -١ وجين السيتوكروم ١أ في الأسماك صغيره الحجم في منطقتي الحامول والرياض كانت اعلي من التي حصل عليها في منطقه سيدي سالم , في حين أن التعبير الجيني لجين الانسولين عامل النمو -١ في الأسماك كبيره الحجم في منطقه سيدي سالم كانت ذو تاثير واضح مقارنتا بالاحجام المختلفه في اماكن الدراسه الأخرى , بينما التعبير الجيني لجين السيتوكروم ١أ في الأسماك متوسطه الحجم في منطقه سيدي سالم كانت ذو تاثير واضح مقارنتا بالاحجام المختلفه في أماكن الدراسه الأخرى . لذا فان التلوث بالمبيدات الحشريه لها تاثير علي التعبير الجيني لجيني الانسولين عامل النمو -١ و السيتوكروم- ١أ لذلك نستطيع ان يستخدم كدلائل في الكشف علي الأسماك المعرضه للتلوث.