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MOLECULAR EFFECT OF AFLATOXIN B1 IN SOME GENE

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Abstract

The PCR reaction was targeted against genes in the rat's lung and liver that were treated at different concentrations of aflatoxin B1. IL-6, IL-2, c-myc and rasp-21, PCR product results for IL-6, IL-2, c-myc and rasp-21 showed high sensitivity and high specificity in immunoglobulin and antioxidant detection. Nuclear isolated from the liver and lung was first introduced as a template and PCR was implemented with each group of primers in the study, an amplicon corresponding to the product size 185 basis points, 330 basis points, 500 basis points, 185 basis points,, 345 beats per inch, and 264 beats per Wear and tear respectively were seen after PCR agarose gel electrophoresis test with three different concentrations in the liver and lung showed that the genetic DNA of the samples was identified and supplemented for IL-6, IL-2, bax F and c-sequence myc and p5 genes 3, rasp-21 and g3pdh are represented by the presence of a single band of product size 185 bp, 330 bp, 222 bp, 500 bp, 185 bp, 345 bp, 264 bp by molecular weight per organic liver and lung as well as per concentration. Sequence alignment using BLAST and BioEdit showed replacement of Transverse ion in IL-6, c-myc, rasp-21 and g3pdh with a join number: JN98643.1, JF76532.1, UI23457.1 and JO96356.1, respectively, in 100% compliance with NCBI, 2026 degree and expect 0.0 and replace the transformation in the genes IL-2, Pax F, and rasp-21 and that this information about the types of mutations detected in the partial liver tissue gene using immuno-primer and antioxidants after exposure to aflatoxin B1 listed in Table 2. After analyzing the sequence of gene results In lung tissue a different type of replacement appeared in the gene analysis. Transversion in the lung genes IL-6, IL-2, bax F, c-myc, p53 and g3pdh is replaced with Sequence ID LN998078.1,. KM340988.1, KS576160.1, QS094532.1, YM547899.1 respectively. Transition alternatives in LM092240.1 IL-2 gene with location sequence ID JH279509.1 in the nucleotide range 8 to 3936 compared with data obtained from gene bank

***Keywords*:** Aflatoxin B1, *Asprgillus flavus*, Mutation.

Introduction

Aflatoxin B1 is a metabolite born and adds food supplies in certain parts of the world. It is produced by *Aspergillus flavus* and related fungi that grow on wrongly stored foods, which require a change from one form to another of AFB1 from using powerful drugs to help cure disease and food use to exo-8,9-epoxide (Nevill *et al*, 2009; Hussain, 2010). The production of aflatoxins produced by many group of similar living things almost the same as *Aspergillus* a naturally happens. *A. flavus* and A. are related to things that feed slowly and weaken other things. Also, there is a group of almost the same living things (Mohammed, 2008). while fungal Aflatoxin are far from other products produced for aflatoxin in agriculture-based products. The main categories of fungal poisonous chemicals include the discussion of fungal metabolites: aflatoxin B1 (AFB1), (Al-Qazzaz , 2012; Xavie *et al.*, 2008) the most commonly known cancer-causing agent, which has long been shown to be poisonous to tiny chemical assembly instructions inside of living things. His name was taken from early work that discovered A. flavustoxins. Aflatoxins are poisonous and among the most known cancer-causing things, after entering the body, aflatoxin B1 chemically treated and used may be treated by liver enzymes for changes that cause reactions from other people or chemicals to medium-sized epoxy compounds to become less harmful such as aflatoxin M1 (Kubiczkova *et al.*, 2012). It can grow mainly in warm, humid and sub-related to areas near the Equator areas, while flavus has been developed in a wide range of temperatures in a supporting structure that changes a chemical with high hydrocarbon content, but many non-poisonous strains do the same, Mycotoxins are produced at the end of the increase stage more and more over time or at the beginning of the immature phase of the mold growth period (Loeffler *et al.*, 2014; Massague, 199), in order to destroy the DNA, AFB1 chemically treated and used is processed with guanine to form some comparisons, Is that these roads (Bandyopadhyay *et al.*, 2006), or Damage to the secondary DNA of the body's parts comes from them, leading to small chemical device made up of smaller parts instructions within living things related to the objects received. Although there are a variety of different types of people and DNA types that are formed in chemically treated poisonous cells, related to things that feed slowly and weaken other things (Al-Azzawi, 2006). The range of poisonous chemicals is controlled by one change related to small chemical device made up of smaller parts instructions with in . These changes are supposed to start from Guan's expression, because all of the aflatoxin killings of almost all, if not all, people happen on this basis (AL-Dujaily *et al.*, 2008).

Materials and Methods

Production of Aflatoxin B1 : The isolate of *A. Flavus* grown on Sabouraud Dextrose Agar (SDA) plates for 3 days at 37 °C after copelet growing the conidia harvested with sterile saline with 0.1 % 80 (Tween® 80) and adjusted to a concentration of 10^7 conidia /ml calculated using haemocytometer. Inoculate 100 ml of liquid medium (YES) in 250 ml flasks with one milliliter volumes of this conidial suspension. The cultures were incubated at shaking incubator with condition 37 °C , 7days. Extraction of Aflatoxin B1 done from biomass according to (Al-Sanat, 2009). Extracted Aflatoxin B1 was subjected using Thin layer chromatography (TLC) through spotted onto a Silica plate heated for 10 minutes at 110 °C and viewing the plates under the UV cabinet at wavelength 366 nm. American Cancer Society ,2007). Then detected the concentration of Aflatoxin B1 sample using chromatography (HPLC) analysis when compared with standard sample.

Experimental Design and method of Injection

Twenty four mice were divided into 4 groups 6 mice for each group as following:

Group I : Mice were treated firstly Intraperitoneally (I.P.) with 25µL of 10% methanol for 14 days and considered as control group, three of them were sacrificed after 14 days.

Group II: Mice were treated Intraperitoneally (I.P.) with 25µL of extracted Aflatoxin B1 75 µg/ml were sacrificed after 14 days .

Group III : Mice were treated Intraperitoneally (I.P.) with 25µL of extracted Aflatoxin B1 150 µg/ml, were sacrificed after 14 days .

Group IV: Mice were treated Intraperitoneally (I.P.) with 25µL of extracted Aflatoxin B1 200 µg/ml (15 µg) for 14 days , were sacrificed after 14 days.

Extraction of DNA from mice Liver and lung

After management of (pulled out or taken from something else) Aflatoxin B1, mouse was (drugs that cause numbness or unconsciousness) and abdomen was opened (straight up-and-down), samples (lung, liver) were pulled and put in petri dish containing body-structure-related solution to remove (remove from a ruling position)/ legally state under oath and connective tissues, (related to shape and structure) changes on the organs were showed and used in the molecular analysis study (Caterina *et al.*, 2008).

The (related to the study of tiny chemical instructions within cells) DNA (pulled out or taken from something else) using Mini Kit (Tissue) /(Geneaid/Taiwan ID: G657k) was made for purifying total DNA and allowing DNA to be easily bound by the glass fiber matrix of the spin column (1). Once cutting up to 30 mg of liver and lung from each animal groups and move (from one place to another) it to a 1.5 ml micro (device that spins something at a high speed) tube and continue to (blend and mix together very well) the sample tissue by grinding, and Create and grow at 60ÂºC for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes At this time, preheat the needed/demanded Elution Buffer (200 Î¼l per sample) to 70Â ºC (for Step 5 DNA Elution). Add 200 Î¼l of complete and total ethanol to the sample lysate and swirl immediately for 10 seconds then let stand for 5 minutes or until the Elution Buffer or TE was soaked up (like a towel) by the matrix, and (device that spins something at a high speed)d at 14.16,000 x g for 30 seconds to elute the purified DNA. The concentration of DNA and DNA purity were shown by nanodrop method. DNA quality was also tested/evaluated by simply carefully studying the DNA by lab chemical gel electrophoresis (Harris *et al.*, 2007).

Increase/ (making something clear) PCR for some liver and lung (tiny chemical assembly instruction inside of living things) (immunological and body-protecting chemical genes): The easy basic reading books sequence were designed depending on the website :www.primer3.com. A specific-PCR easy books/basic reading books with an amplicon size wear listed to forward and reverse (the (easy book/basic reading) set was supplied by IDT (Integrated DNA Technologies company, Canada). The sound-related program was one cycle at 94 Â°C for 1 min; 35 cycles of 94 Â°C for 1 min, 63 Â°C for 1 min, 72 Â°C for 1 min and 72 Â°C for 10 min, using the Mastercycler (Eppendorf). The louder/clearer product was subjected to 2% lab chemical gel electrophoresis, and saw (in your mind) under UV (Imagemaster VDS, Pharmacia (the science of living things), USA) after staining with Ethidium bromide (Hayes *et al.*, 2006).

Table 1: Immunological and antioxidant genes used in the study.

Primer	Product Size	Tm	Sequence 5′-3′	GC%
IL-6 Interleukin6	**185 bp**	**57**	F 5′-TGG AGC CCC TGA AGA AGA G-3′ R 5′-AAG TGC GTT GTG CGG TAG C-3′	**50**
IL-2 Interleukin2	**330 bp**	**55**	F 5′-CAA GCC GGG AGA ACA GGG TA-3′ R 5′-CCC ACC GAA CTC AAA GAA GGC-3′	**50**
c-myc	**500 bp**	**55**	F 5′-TGA CGA GAC CTT CGT GAA GA-3′ R 5′-ATT GAT GTT ATT TAC ACT TAA GGG T-3′	**50**
rasp-21	**345 bp**	**56**	F 5′-ACT TGT GGT AGT TGG CCC T-3′ R 5′- TCC CCA GTT CTC ATG TAC TG-3′	**50**

Positive PCR product samples of lung and liver for each immunological and body-protecting chemical (tiny chemical assembly instruction inside of living things) were sent for sequence analysis; nd 25 Âµl (10 pmol) from the forward (easy book/basic reading). The samples were treated with AB13730XL APPLIED BIOSYSTEMS machine in national instrumentation centre for (related to surrounding conditions or the health of the Earth) management NICM/USA company online at (http://nicem.snu.ac.kr/main/?en_skin=index.html). The result of the sequence analysis was analysed by blast in the National Centre (science that uses living things to improve the Earth) Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and (story of a person's life) Edit program in order to measure the change effect of Aflatoxin B1 in the immunological and body-protecting chemical (tiny chemical assembly instruction inside of living things) using the clearly particular easy reading.

Statistical analysis

Data of sequencing were analysed using the model procedures of NCBI. The differences of values of the investigated were assessed by analysis of variance using SAS version 7.5, (difference p<=0.05 and 0.001) (Abed, 2012).

Results and Discussion

The (related to the study of tiny chemical instructions within cells) DNA extraction from fresh liver and lung tissue samples of mice used extraction kit DNA Mini Kit (liver Tissue and lung)/ (Geneaid (the science of living things) Ltd) it was simple and fast DNA extraction rules of conduct. The results showed the DNA extraction was performed

successfully from the fresh tissue of the mice liver and lung for each Aflatoxin B1 injection the DNA (measuring something exactly) data received/got by nanodrop method (AGTGAN/avans company/tiwan), which showed the concentrations and the purity of the different sample included in the study ranged from 264 to 3551.5 ng/Î¼l, according to the results received/got the DNA samples were total/totally/with nothing else mixed in and good to do the PCR way of doing things. After DNA purification, it used for PCR increase/(making something clear), repeated runs of PCR were done to increase the DNA (pulled out or taken from something else) from samples optimization Easy books reading books concentration and DNA concentration were (sang, danced, acted, etc., in front of people) for these samples, many optimization reactions were sang, danced, acted, etc., in front of people on this easy books reading books set, PCR optimization runs repeated many times. The best concentration of the easy books/basic reading books was 10 p.mol which was best for increasing the samples. All the positive samples were used together in the same PCR run plus the control results were gotten when moved on lab chemical gel electrophoresis.

PCR For immunological and body-protecting chemical IL-6, IL-2, bax F, c-myc, p53, rasp-21, and g3pdh tiny chemical assembly instructions inside of living things PCR program was successfully used in this study, which designed to increase samples it was found that 25Î¼l was the best reaction sound level for this study. In this study, the PCR reaction was targeted against the (tiny chemical assembly instructions inside of living things) in liver and lung of mice treated with Aflatoxin B1 different concentrations. The results of PCR products of IL-6, IL-2, c-myc, and rasp-21 easy books/basic reading books showed high sensitivity and level of detail in detecting immunological and body-protecting chemical, DNA separated far from others from liver and lung were first used as example that should be copy and PCR carried out with all set of easy books reading books design in the study, an amplicon going along matching up to Product Size 185 bp, 330 bp, 222 bp, 500 bp, 185 bp, 345 bp, and 264 bp (match up each pair of items in order) wear seen after lab chemical gel electrophoresis PCR was tested with three different concentrations in liver and lung showed that the related to the study of tiny chemical instructions within cells DNA of samples were recognized and completing to IL-6, IL-2, c-myc, and rasp-21 sequence and represented by presence of single band Product Size 185 bp, 330 bp, 222 bp, 500 bp, 185 bp, 345 bp, and 264 bp match up each pair of items in order molecular weight to each organ liver and lung and also to each concentration 75, 150, 200 Âµg/ml.

The positive results of each tiny chemical assembly instruction inside of living things in the different concentrations may be attributed to the response of stress condition by Aflatoxin B1 will cause either cell cycle arrest or cell death (Hayes *et al.*, 2006; Ali, 2012), a fungal Aflatoxin B1 produced by *A. flavus* related to how the body uses food activated by the liver cytochrome p450 system into a highly (causing reactions from other people or chemicals) electrophilic intermediate, AF 8, 9-epoxide, that can form DNA moves toward the body that play a big part in causing changes in rats hepatocellular cancer-causing process (Ali, 2010).The presence and indication of g3 pdh tiny chemical assembly instruction inside of living things may be related to its important conjugation of causing reactions from other people or chemicals xenobiotic metabolites with glutathione is an important step in detoxification and is agreed by g3pdh tiny chemical assembly instruction inside of living things, an overload of xenobiotic may use reduce glutathione through conjugation activities, there by giving to oxidative stress (K/DOQI Workgroup. K/DOQI, 2005). Also, previous study reported that decreased liver-related expression of the studied tiny chemical assembly instruction inside of living things, as followed in rabbit exposed to Aflatoxin B1, could limit the liver-related tissue's and lung ability to conjugate (causing reactions from other people or chemicals metabolites (Al-Qazzaz, 2012).

Sequencing Analysis

Positive PCR products were purified in order to use the samples in putting in correct order. The sequences were carefully studied to detect the presence of changes in the PCR product of the samples of liver and lung tiny chemical assembly instructions inside of living things and compared with the international (computer file full of information on the National Center For science that uses living things to improve the Earth Information (NCBI), then the changes were scored, and compared with the sequence of the control.

Change detection by the DNA putting in correct order way of doing things

putting in correct order of IL-6, IL-2, bax F, c-myc, p53, rasp-21, and g3pdh tiny chemical assembly instruction inside of living things in lung and liver organs exposure to concentration 75, 150, 200 Âµg/ml were performed and to confirm the identification of change during the exposure time to glioxin, Sequences matching up in a straight line using BLAST and BioEdit showed type of Change, scored and the matching up in a straight line applied, expected changes were shown in table 2 and 3. The expected changes were found after the analysis of the sequences Data in the sample treated with Aflatoxin B1 200 Âµg/ml in liver and lung while in the sample treated with 75 and 150 Âµg/ml theirs no change, this may be related to the unable to be harmed response system against this two applied concentrations (Kubiczkova *et al.,* 2012).

Sequences matching up in a straight line using BLAST and BioEdit showed Transverse ion substitution in IL-6, c-myc, rasp-21, and g3pdh with rising up number : JN98643.1, JF76532.1, UI23457.1and JO96356.1 match up each pair of items in order 100% compatibility with NCBI, score 2026 and expect 0.0 and Change from one thing to another substitution in IL-2, bax F, and rasp-21 tiny chemical assembly instruction inside of living things all the information about the Types of changes detected in partial liver tissue tiny chemical assembly instruction inside of living things using immunological and body-protecting chemical (easy book/basic reading) after exposure to Aflatoxin B1 listed in Table 2. After the analysis of the sequences results of the tiny chemical assembly instructions inside of living things in the lung tissue different type of substitution appeared in the analyses genes as listed in table (3). There are Transversion substitution in the genes of lung IL-6, IL-2, bax F, c-myc, p53, and g3pdh with Sequence ID LN998078.1,. KM340988.1, KS576160.1, QS094532.1, YM547899.1 respectively. Transition substitution in LM092240.1 IL-2 gene with Sequence ID JH279509.1.

Table 2 : Types of mutations detected in partial liver tissue gene using immunological and antioxidant primer after exposure to 200 µg/ml Aflatoxin B1.

Sequence ID	Range of nucleotide	Nucleotide	Location	Type of substitution	Name of gene
JN98643.1	76 to 1217	T>G	227	Transversion	**IL-6**
JC65480.1	30 to 700	G>A	690	Transition	**IL-2**
JF76532.1	19 to 489	C>A	639	Transversion	**c-myc**
GH23579.1	70 to 1160	G>A	634	Transition	**p53**
UI23457.1	**120 to 2013**	**T>A**	**789**	**Transversion**	**rasp-21**

Table 3 : Types of mutations detected in partial lung tissue gene using immunological and antioxidant primer after exposure to Aflatoxin B1.

Sequence ID	Range of nucleotide	Nucleotide	Location	Type of substitution	Name of gene
LN998078.1	65 to 2310	G>T	143	Transversion	**IL-6**
LM092240.1	7 to 985	C>T	568	Transition	**IL-2**
KS576160.1	99 to to 2012	T>G	165	Transversion	**c-myc**
YM547899.1	**67 to 543**	**G>T**	**280**	**Transversion**	**rasp-21**

The putting in correct order analysis show different type of change that's may be related to the direct role of Aflatoxin B1 in the cause change through enters the center of a cell or atom and cause oxidative damage, by that/in that way decreasing the number of times something happens of cancer and unable to be harmed stopping/preventing (actions or feelings) (Loeffler *et al.*, 2012). Many cancer-causing substances including mycotoxin damage DNA (Mohammed 2008). The Aflatoxin B1 destructive effects may be caused from (related to tiny chemical assembly instructions inside of living things) material damage through different (machines/methods/ways), eating of Aflatoxin B1e resulted in a big drop in the DNA and RNA contents in the liver and lung that's lead to cause different type of changes (Loeffler, 2014), especially in the studied (tiny chemical assembly instruction inside of living things) during the present study. Aflatoxin B1 covalent binding with the nucleic acids happens within minutes of Aflatoxin B1 management this binding results in a steep and dangerous decrease in both DNA and RNA combination the thing that's given of each DNA move toward the body to the induction of change is not clearly shown induction of changes during DNA answer/copy in the lung and liver (American Cancer Society 2007).

Results shown that the Aflatoxin B1 may lead to part of the move toward the body is involved in the G-T to lesser extent G-A changes at the site of the damage to a body part Aflatoxin B1 caused changes, including the G-T transversion at the second and the third position of the codon 247(Abed, 2012). Aflatoxin B1 puts into action part of its immuno holding blocking effects through these lymphokines. The effect of glioxin in the interleukin watched/followed a decrease in the (written version of spoken words) al levels of IL-2 in these thymocytes lead to changes in specific genes (Sambrook *et al.,* 1989).

In the present study, change of in the experiments tiny chemical assembly instructions inside of living things was down-controlled in extremely pale or white in color mice exposure to Aflatoxin B1 and this increase type of change (tiny chemical assembly instruction inside of living things has been attributed to the superoxide negatively-charged ions piled up within the mitochondria, this way leading to an oxidative stress and in that way interfering with the cellular processes (Bandyopadhyay *et al.*, 2006). The animals receiving dose of Aflatoxin B1 with high concentrations compared to those receiving low concentration, an indication of another type of change Aflatoxin B1 caused mutagenicity and hepatocarcinogenecity by stopping cytochrome P450 in the liver and also induction of body-protecting chemical enzymes (American Cancer Society 2007). From The above findings we decided presence of possible change role related to Aflatoxin B1 in some liver and lung tiny chemical assembly instruction inside of living things.

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