ASSESSMENT OF AFLATOXINS IN SOME FOODS AND ITS RELATION TO HEPATIC DISEASES

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ABSTRACT

Humans are exposed to hepatocarcinogenic aflatoxins (Afs) through ingestion of contaminated foods as a result of poor storageful of susceptible grains or eating foods contaminated with aflatoxins animals and vegetables. This work aimed at evaluating the effect level of total (Afs) on human health. Study samples ninety persons divided into three groups (each /30 human). Group (1) no suffering liver disease as normal or negative control. Group (2) patients input (El Raghi hospital in Assuit) are suffering of liver inflammatory disease (HI). Group (3) patients input/or output (El Raghi hospital in Assuit) are suffering of hepatocellular carcinoma (HCC). Random samples were obtained from some foods (chicken egg; chicken, duck and rabbit liver) purchased from local market in Assuit. Total amount of aflatoxins was determined by using high-performance liquid chromatography (HPLC) instrumental.

This study used 24 h recall and food frequency questioners for these groups to determination the type and amount of food which were intake. In serum humans measured total aflatoxins by used the enzyme-linked immunosorbent assay (ELISA) kits and ELISA technique. In addition oxidation tests are carried out for the studied individuals by using colorimetric kites to determine liver function (Alanine transaminase (ALT), Aspartate transaminase (AST), Total protein (TP),albumin (Alb), Bilirubin (Total, Direct), alkaline phosphatase (ALP), Glutathione peroxidase (GPX) and Malondialdehyde (MDA) by used spectroscopy instrument.

Results indicated that were significant increase in liver function and biomarker antioxidants (GPX, MDA). Study concluded that these alterations in liver functions could be related to the development of liver damage in

response to significant dose of Aflatoxins and this work aimed at evaluating the effect level of total (Afs) on human health.
Key words: Aflatoxins- health of humans- liver disease.

INTRODUCTION

WHO (2014) reported that there are lots of environmental toxins in our air, water and/or food supply affecting of humans health. Food contamination is a public health problem that is monitored worldwide. Fehizardo and Camara (2013) found that care in food preparation and storage is so serious to avoid intake of various microorganisms and their toxins.

Liu & Wu (2010) showed that aflatoxins are a group of approximately 20 related fungal metabolites. The four major aflatoxins are known as B1, B2, G1, and G2. Aflatoxins B2 and G2 are the dihydro-derivatives of the parent compounds B1 and G1.Aflatoxins refer to serious health, economic and agricultural problems in developing countries Afum *et al.* (2016)

The biosynthetic pathway of aflatoxins consists of 18 enzymatic steps for conversion from acetyl-CoA, and at least 25 genes encoding the enzymes and regulatory pathways have been cloned and characterized (Yabe and Nakajima, 2004).

Wild *et al.* (2002) indicated that aflatoxins (AFL) contaminate food during storage, production and processing. Due to their high toxicity and carcinogenic effects, they have long been suggested as possible an etiologic agent of hepatocellular carcinoma (HCC). Jaimez *et al.* (2000) the level of toxicity associated with aflatoxin varies with the types present, with the order of toxicity being AFTs-B1 > AFTs-G1 > AFTs-B2 > AFTs-G2. Reddy *et al.*

(2010) who said that excreted aflatoxins metabolites were related to have an increased risk of HCC.

The adverse health effects of aflatoxins can be categorized as either acute or chronic. Acute aflatoxicosis occurs when moderate to high levels of the toxins are consumed and may result in hemorrhage, acute liver damage, edema of the limbs, alteration in digestion (absorption and/or metabolism of nutrients), high fever, vomiting, swollen livers and possibly death Verma (2004).

Chronic aflatoxicosis is results from ingestion of low to moderate levels of aflatoxins and the effects are usually subclinical and difficult to recognize CDC (2004).

WHO (2014) reported that liver cancer is the sixth most common cancer worldwide, with 782,000 new cases diagnosed in 2012. It is the second most common cause of death from cancer and is more common in men than women. Hepatocellular carcinoma (HCC) accounted for 70%-90% of primary liver cancers, making it the third leading cause of cancer-related deaths worldwide El-Serag *et al.* (2007).

This study carried out on aflatoxin in some food that may be the cause liver diseases in humans and relation between Aflatoxin in foods and HCC in humans.

MATERIALS, SUBJECT AND METHODS

Materials:

- Foods: chicken egg; chicken, duck and rabbit liver, purchased from local market in Assuit.

- Kits and chemical for analysis will obtained from El Gomhoria com., Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Subjects:-

90 studied groups (30 normal; 30 inflammation liver and 30 HCC) were obtained from El Raghy Hospital for liver diseases.

Methods:

- Dietary Assessment for patient by measure the quantity of food items consumed over one day period "twenty four-hour recall" The second methods included the dietary pattern by a questionnaire of food frequency method according to NNI, 2006 and then analyze quantity for some Aflatoxins in some foods by using HPLC according to Sirhan *et al.*, (2011), Determinate liver function (enzymes, natural antioxidant and lipid peroxidation) by spectrometer according to (Young, 2001; Tietz, 1990 & 1995; Moss, 1982; Beutler *et al.*, 1963 and Uchiyama & Mihara, 1978) respectively and Aflatoxins albumin adduct using ELISA Kit for patients by using sera according to Zain (2011).
- Statistical analysis: Data were statistically analyzed of variance “ANOVA” test at ($P \leq 0.05$) according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Characterization of Groups:

Table (1): Demographic data between different study groups

Groups / Sample		G1 Normal (n=30)	G2HCC (n=30)	G3HI (n=30)
Average age "years" Range of Age:		42.09±13.10 (20.0-70.0)	58.83± 7.60 (45.0-71.0)	63.36±11.80 (35.0-89.0)
Gender	Male	13(43.33%)	25(83.3%)	23(76.7%)
	Female	17(56.67%)	5(16.7%)	7(23.3%)
Place of treatment	Inpatient	0	8(26.67%)	25(78.123%)
	out patient	30 (100%)	22(73.33%)	5(16.67%)
Area	Urban	16(53.33%)	9(30.0%)	14(46.67%)
	Rural	14(46.67%)	21(70.0%)	16(53.33%)

Demographic data of study groups with mean age 42.09 years in normal group and 58.8 in HCC group and there were mean age 63.36 years in HI group. These results in table (1) were harmony with Afum. *et al.* (2016) they found that the most liver diseases cases were 70% for participants aged 25–34 years.

Table (2): Value of Aflatoxin types in eggs and livers (chickens, rabbits and ducks)

Samples / Parameter	Aflatoxins maximum level allowed	Liver chicken (n=5)	Liver rabbit (n=5)	Liver duckling (n=5)	Eggs (n=5)
AflatoxinB1 (ppb)	-	Negative	Negative	Negative	Negative
Aflatoxins B2 (ppb)	-	22.2±0.2	26.3±0.5	22.3 ±1.0	Negative
Aflatoxins G1 (ppb)	-	18.8± 2.0	18.9 ±1.9	18.8±1.05	Negative
Aflatoxins G2 (ppb)	-	13.5± 1.4	13.5±1.09	13.7±2.01	Negative
Aflatoxins total (ppb)	20 ppb	54.6± 2.5	58.6± 3.0	54. 8 ±3.8	Negative

Table (2) showed that the value of Aflatoxins in eggs and livers of chickens rabbits and ducks. There are no Aflatoxins B1 found in eggs & liver,

Also about Aflatoxins B2, G1, G2 and total they are found in liver but negative in eggs which present in Assuit market. About Aflatoxins B2, G1, G2 and Aflatoxins total there are positive in liver (ducks, rabbit, and chicken), there are higher amount of total aflatoxin in all types of liver than maximum level allowed. Bryden (2012) reported that the contaminated animal feed is the major cause of exposure of these mycotoxins to animals and therefore ultimately to humans. Negative total aflatoxin in the different types of eggs these results agree with Jia. *et al.* (2016) found that combined aflatoxin (AF) and zeralion (ZEA) contamination showed synergistic effects for decreasing egg production, feed intake, feed conversion ratio and eggshell strength in birds.

Table (3): Food intake of different patients from some food contain aflatoxins, mean ± SE (g /d)

Groups / Sample	Normal Group	HCC patients group	HI patients Group	*P* value
Chicken liver (g/d)	13.4±18.6	43.4 ±7.3	42.67 ±4.8	*P*<0.000
Rabbit liver (g/d)	15.9 ±1.9	15.3 ±2.0	14.5 ±2.8	*P*= 0.910
Duck liver (g/d)	6.6 ± 1.4	7.6 ±2.0	12.2 ± 1.1	*P*<0.033

Table (3) shows food intake of different study groups from foods contains Aflatoxins. There were highly significance difference (p<0.000) about chicken liver intake between different groups, also there were significance difference (P<0.05) between different groups about duck liver intake; but there were no significance difference between groups received rabbit liver (P>0.05). Food consumption pattern has dramatically changed in some countries. Socio-cultural factors such as religion, beliefs, food preferences, gender discrimination, education and women's employment all have a noticeable influence on food consumption patterns in this region. Iqbalab *et al.* (2014) reported that the consumption of chicken meat and eggs are increasing due to its availability on reasonable prices. However, the considerable finding levels of AFs, in chicken meat and eggs are alarming for the health as well as the economy of the country. Therefore, urgent steps should be taken to monitor and control these toxins in chicken meat products. The strict permissible limits should be implemented to avoid fungal contamination.

Table (4): Glutathione Px, Aflatoxins and MDA in studied groups:

Parameter	Cut of point	Normal Group	HCC group	HI Group	P value
Glutathione GPx mg/dl	49	33.94±4.8	20.9±4.4	15.2±1.2	P<0.000
MDA µmol/ml	31.9	31. 1±0.4	42. 2±0.5	55.3 ±0.7	P< 0.013
AFB1_adduct µg/L	0.3_24.3	18.2±3.4	155.±11.9	355.6±24.2	P< 0.000

Table (4) showed that there were significant increase of MDA value between normal and patient groups (HCC and HI) while, noticed was a decrease in GPx value. High AFB1 adduct levels in serum of HCC and HI groups of study participant's confirm chronic dietary exposure to aflatoxin. The results were so may be due to AFs caused increased oxidative stress. In present study there was decrease in GPx enzyme in patients with HCC & HI. The lower level of the antioxidant enzyme glutathione peroxidase in patients with cirrhosis indicated a severe oxidative stress explained by its utilization in scavenging the free radicals, this results agree with Sineque *et al.*, (2017) that excessive generation of free radicals leads to inactivation of enzymes and decreased level of GPx enzyme activity as a result of the impaired GPx activity in patients.

Table (5): Liver function parameters in studied groups

Parameter	Cut of point	Normal Group	HCC patients group	HI patients Group	*P* value
AST (U/L)	Up to 38	30.3± 1.7	69.8±11.3	106.7±13.5	0.000
ALT (U/L)	Up to 40	28.5± 1.6	54.9± 9.5	73.2± 8.2	0.002
GGT (U/L)	Up to 50	19.5±2.1	44.1±7.1	92.2±12.6	0.000
T.BIL(Umol/L)	Up to 21	14.9±0.5	24.2±3.8	57.1±14.4	0.002
D.BIL(Umol/L)	Up to 4.25	3.0±0.2	12.6±2.2	41.4±12.2	0.001
ALP (U/L)	40 _180	151.4±9.2	177±31.6	199.5±22.9	0.344
T.protein g/L	64_83	65.7±1.3	63.6±1.9	73.6±2	0.0003
Albumin g/L	48_54	40.9	28.3	32.3	0.000
Globulin g/L	16-29	24.9±1.9	35.4±2.2	41.2±1.6	0.000

The vast majority of individuals with abnormal liver biochemistry had the parameter evidence of liver diseases. Liver disease is often reflected by biochemical abnormalities of liver function. Table (5) showed liver function in the different study groups. There were highly significance difference ($P<0.000$) between different groups in each of AST, ALB and TP and there were moderate significance difference ($P<0.001$) in each of ALT, T. Bilirubin and D. Bilirubin. There are not significant differences between normal group and other groups in ALP value. These result agree with Ali and Nawaz, (2017) who recorded that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function is specific for liver disease, In this present study AST, ALT, GGT and ALP levels in HCC and HI groups often are higher than AST level in normal groups.

CONCLUSION

In conclusion, the results of the present investigation exposure to low concentration of aflatoxin consumption of food for long time are cause HI and HCC. Consequent upon this, humans should avoid the contaminated food and using storage ways for foods healthy, protect animals from contaminated feed by helping the government.

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تقييم الافلاتوكسينات في بعض الاغذيه وعلاقتها بأمراض الكبد

[٣]

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المستخلص

يتعرض الإنسان لإصابة الكبد بالأفلاتوكسينات من خلال تناول الأطعمة الملوثة نتيجة للتخزين السيئ للحبوب الحساسة أو تناول الأطعمة الملوثة بالأفلاتوكسين سواء من مصدر حيواني أو نباتي. أجريت هذه الدراسة لتقييم الافلاتوكسينات في بعض الاغذيه وتقييم علاقتها بسرطان الكبد في الإنسان حيث جمع عينات من البيض واكباد (الدجاج، الأرانب، البط) من السوق المحلي لمدينه أسيوط. وجد أن نسبه الافلاتوكسينات الكلية في جميع العينات مرتفعه عن الحد المسموح به من منظمه الصحه العالميه باستخدام جهاز كروماتوجرافيا السائل فائق الاداء وكانت اعلي نسبه في اكباد الدجاج يليها اكباد الأرانب ثم اكباد البط.

تم سحب ٣٠ عينه دم من مرضي سرطان الكبد ٣٠ عينه دم من مرضي أمراض الكبد من مستشفي الراجحي الجامعي باسيوط ٣٠عينه دم من اصحاء لايعانون من أمراض في الكبد. تم إجراء الاختبارات البيوكيميائيه لانزيمات الكبد ومضادات الأكسدة الطبيعية وبيروكسيد الدهون و قياس مستوي الافلاتوكسين باستخدام جهاز الاليزا والتقييم الغذائي لجميع المجموعات. تم اكتشاف زيادة واضحة في إنزيمات الكبد لدي مرضي سرطان الكبد مع زياده مستوي الافلاتوكسين في دم المرضي. وقد تودي إلي سرطان الكبد.

أوضحت الدراسة أن للسموم الفطرية أثراً سلبياً علي صحة الإنسان والحيوان وتوصي الدراسة بأن تناول الغذاء الصحي وتوفير وسائل وعي للمستهلكين قد يودي إلي التقليل من تلك الآثار علي صحة الإنسان والحيوان.

الكلمات الدالة: (الأفلاتوكسينات – أمراض الكبد – صحة الإنسان).