ANTIOXIDANT ACTIVITY OF ASCORBIC ACID AGAINST AFLATOXIN IN CONTAMINATED NUTS ON RATS

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ABSTRACT

The current study was undertaken to analyse the effect of aflatoxin on protein and fat in nuts (almonds and walnuts) and to evaluate the protective effect and antioxidant activity of ascorbic acid in aflatoxin contaminated nuts. Almond and walnut were stored for six months at high temperature and humidity. The results showed that aflatoxin B1, B2, and G2 were higher in walnuts whereas G1was higher in almond after six months storage. The results suggested that protein and fat decreased significantly, while highly significant increase was observed in moisture content. Vitamin C is an important non enzymatic antioxidant and a free radical scavenger, thereby prevents the production of electrophilic metabolites. The lowering effect of vitamin C in aflatoxin contaminated nuts was investigated in experimental rats. Fifty white albino rats were randomly classified into five groups: (1) control negative (-ve), (2) 4%almond in basal diet, (3) 4%walnutsin basal diet, (4) 4%almond with vitamin C in basal diet and(5) 4%walnuts with vitamin C in basal diet) groups. Consumption of almond and walnuts contaminated with aflatoxin alone or in combination with vitamin C showed slight increase in final weight, body weight gain and food intake as compared to control negative group. Administration of vitamin C with almond or walnuts reduced the higher level serum ALT, AST, ALP and GT but liver cholesterol, total lipid, triglyceride and glycogen were within normal values. Consumption of walnuts in combination with vitamin C showed significant increase in serum total bilirubin, creatinine and urea compared to control negative group but lower as compared to consumption of walnuts only and showed normal value of serum uric acid. This study proved that vitamin Clowers the effect of aflatoxin.

Keywords: Almond, Walnuts, Aflatoxin, Vitamin C, Albino Male Rats.

INTRODUCTION

Nuts contain high levels of protein, fat and dietary fibers, which in association with their pleasant flavor and convenience, have led to the recommendation that they should be an essential part of healthy diet. This was recently endorsed by allowance of a qualified health claim for a relationship between the consumption of nuts and reduced risk of coronary heart disease (CHD) by the Food and Drug administration (FDA ,2003). Almonds *(Prunes Amygdalus L)* have several Health Benefits. Regular consumption of almonds helps to increase the level of high density lipoprotein and reduce the level of low density lipoproteins. This balance is vital to a healthy cholesterol level, and reduction of LDL (bad cholesterol) is always a good thing to reduce heart attack risk. Almonds are packed with vitamins, minerals, protein ,fiber and are associated with number of health benefits .just a handful of almonds, approximately one ounce, contains one-eighth of our necessary daily protein(Minsh, 2014)Almonds are a source of Vitamin E, copper, magnesium, and high quality protein. Almonds also contain high levels of healthy unsaturated fatty acids in addition to a lot of bioactive molecules (such as fiber, Phytosterols, vitamins, other minerals, and antioxidants)

which can prevent cardiovascular diseases (Brufau *et al*, 2006).Almonds contain riboflavin and L-Carnitine, nutrients that boost brain activity and may also reduce the risk of Alzheimer's disease. Almonds reduces osteoporosis, boosts immune function, low energy and weight gain (Herrington, 2012). Walnuts contain number of neuro-protective compounds, and contain the amino acid L- Arginine which offers multiple vascular benefits to people with heart disease (Bahorun *et al*., 2006) The walnut is classified as a strategic species for human nutrition and is included by Food and Agriculture Organization of United Nations (FAO) in the priority list of plant based nutrition (Aryapak and Ziarati 2014). Nuts susceptible to fungal (mold) infection, especially by aflatoxin and considered as a high risk commodity due to the contamination of aflatoxin. Aflatoxins are highly carcinogenic, immunosuppressive agent highly toxic and fatal to humans and animals particularly affecting liver and digestive tract. Aflatoxin is a potent human carcinogen It is a naturally occurring toxic metabolite produced by certain fungi (Aspergillus flavus),a mold found on food products' such as corn and peanuts butter(Felicia, 2004) Glutamine aflatoxins are very powerful dangerous toxins, highly carcinogenic, immunosuppressive agents, highly toxic and fatal to humans and animals particularly affecting liver cirrhosis

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and cancer and digestive track. Its toxic metabolites produced by different species of toxigenic fungi are called mycotoxins. At least 14 different types of aflatoxin are produced in nature (Wagacha and Muthomi 2008). Mycotoxins include the most widely studied aflatoxins B_1 (AFB$_1$), B2 (AFB$_2$), G1 (AFG$_1$) and G_2 (AFG$_2$). These mycotoxins are produced as secondary metabolites mostly by some species belonging to Aspergillus flavus section, when growing on a variety of food products (Rodrigues *et al.*,2012). Aflatoxins B_1 (AFB1) is considered as the most dangerous toxic metabolite because of its hepatotoxic, teratogenic immunosuppressive and mutagenic nature. AFB1 is the main aflatoxin found in nuts, and as it is dangerous for health, the European Union (EU) has set a maximum level at 8 µg/kg in samples (FAO, 2004; Wesolek, and Roudot, 2014). Bad storage condition especially moisture content above 12% and temperatures greater than 70° F can also contribute to fungal growth and increase the risk of aflatoxin contamination. 25% of the food crops in the world are affected by mycotoxins. Beans, rice, corn, cottonseed and peanuts are the crops most at risk of being contaminated by aflatoxins(Wang and Liu, 2006; Shadbad *et al.*, 2012) Aflatoxins metabolize to epoxides which bind to guanine in DNA, and lead to lipid peroxidation by robustly generating reactive oxygen species (ROS) and release mutagenic malonaldehyde as well as lead to direct cell injury (Jubeen *et al.*, 2012).The significant reduction in vitamin C level as well as in the activities of glutathione metabolizing enzymes in kidneys of Aflatoxin1 administered rat could be responsible for increased lipid peroxidation observed during aflatoxicosis (Abdel-All *etal.,*2008).Consumption of Aflatoxin contaminated food may lead to nutritional deficiency. Moreover species susceptible to aflatoxin mainly depends on its liver detoxification systems, genetic, age and other nutritional factors (Wild *et al.*, 2000). Ascorbic acidis the water soluble antioxidant, which can protect the body from damage caused by free radicals that can be generated during normal metabolism as well as through exposure to toxins and carcinogens (Rafighi *et al.*,2011).In mammalian cells, vitamin C serves as cofactor for reactions that require reduced iron and /or copper metal co -enzymes. Another important indirect function of Vitamin C is its ability to regenerate other biologically important antioxidants such as I glutathione and vitamin E into their reduced state. The aim of this study is to find the effect of improper storage in aflatoxin contamination of nuts and to investigate the effect of vitamin C against aflatoxin contamination

MATERIALS AND METHODS

Materials: Almonds and walnuts were purchased from local market in Riyadh; Saudi Arabia. Vitamin C was purchased from Sigma Chemical CO., in Riyadh. All chemicals , solvents and biochemical Kits were purchased from Glasgow CO. Fifty white albino male rats (Sprague Dawley strain) weighing 180±5g were provided from experimental animals center in Medicine Collage of King Saud University in Riyadh.:Rats were randomly classified into five groups (10rats in each) as❀Control negative group fed on basal diet only (-ve). ❀Almond group: fed on basal diet with 4% aflatoxin contaminated almond. ❀Walnuts group: fed on basal diet with 4% aflatoxin contaminated walnuts. ❀Almond with vitamin C group: fed on basal diet with 4% aflatoxin contaminated almonds and vitamin C by stomach tube.❀Walnuts with vitamin C group: fed on basal diet with 4% aflatoxin contaminated walnuts and vitamin C by stomach tube.

Animal adaptation: Rats were placed for an adaptation period of one week and were fed on basal diet to allow them to adjust to the new environment. Rat were individually housed in stainless steel cages at room temperature about 25±2Cº with water bottles under hygienic condition and fed diet *ad libitum.*

The basal*:* diet consists 140g casein (83%protein),100g sucrose,50g corn oil,50g cellulose,35g minerals ixture,10g vitamin mixture,1.8g L cysteine, 2.5 g choline chloride, and the remainder 610.6g corn starch. The basal diet was formulated according to (NRC, 1995)

Samples: Almonds and walnuts samples (100g of each) were analyzed before   treatment and were stored in polyethylene bags (200g of each nut) under room temperature at 55% humidity for 6 months.

Methods:

Chemical analysis: Moisture, ash, crude fat, crude protein and crude fiber were determined by standard method of A.O.A.C. (2012). Nitrogen Free Extract (NFE) representing the total carbohydrates was calculated by subtracting the sum of the percentages of moisture, crude protein, crude fat, ash and crude fiber from 100. Nitrogen was determined by Kjeldhal analysis, multiplied by 6.25 and reported as protein. The moisture content of nuts samples were measured using drying oven. The daily food intake and weekly body weight were recorded. Feed efficiency ratio (FER) was calculated according to (Bhilave *et al*., 2012).After the experimental period 45 days) blood and liver were collected. Serum Aspartate (AST) and Alanine Amino Transferase (ALT).Serum Alkaline Phosphates (ALP) and Gamma Glutamyle Transferase enzymes were estimated by using Spectrophotometer according to method described by (Sahoo *et al*., 2014). Serum total bilirubin, creatinine, urea and uric acid were estimated according to methodology adopted by (Sirajwala *et al*., 2013). Serum cholesterol (CHO), triglycerides (TG), and high density lipoprotein cholesterol (HDL-$_C$) were determined. In

addition, liver glycogen, triglyceride (TG), total lipids and cholesterol were determined according to the method prescribed by (Jun *et al*., 2008).

Aflatoxin Analysis: Aflatoxin B1, B2, G1 and G2 were chemically estimated as described by (Ostadrahimi *et al*, 2014; Amiri *et al*., 2013) according to A.O.A.C, (2007) specifications. Samples were analyzed by the ELISA (enzyme-linked immune sorbent assay), using a monitoring scheme consisting of enzyme-linked immune sorbent assay (ELISA) for rapid screening, high performance liquid chromatography (HPLC) for quantification and LC–mass spectrometry (MS) for confirmation. Evaluation of ELISA data as well as the AF concentration was performed using the software program (Euro Clone S.P.A: Italy). Almond and walnuts were crushed into powder using a Kenwood grinder (Mainland, China) into fine particles and fed to rats as 4 % of the diet. Vitamin C was given to rats at dose 250 mg/kg body weight in 5.0 ml of the vehicle by stomach tube all over period of the experiment(45)days)

Statistical Analysis: Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests (Abo- Allam,2003).

RESULTS

Figure (1A) depicts the gross chemical composition of almond and walnuts. The higher levels of protein, fiber and carbohydrate were found in almonds, while walnuts contain very high levels of fat and also found to be rich in protein. Data in fig 1 B shows significant decrease in fat, protein, fiber and carbohydrate respectively in contaminated almonds and walnuts while moisture content increased in both nuts. After six month of storage, determination of aflatoxin in walnuts was higher than in almonds. The level of aflatoxin B1, B2, G1 and G2 in almond was 593.71±12.9, 582±12.84, 232.14±5.24 and 69.77±7.05 respectively, but in walnuts these levels were 992.52±73.45, 49.28±0.0.38, 81.33±11.02 and 677.11±17.97respectively(figure 2).

The effect of consumption of aflatoxins contaminated nuts on body weight gain and FER were illustrated in table1. Consumption of almond and walnuts alone or in combination with vitamin C showed that the values of final body weight, body weight gain and food intake were within normal values of control negative group while significant increase in food efficiency ratio (p<0.05 &0.01) as compared to control negative group was observed (table1). Consumption of aflatoxins

contaminated almonds and walnuts by experimental rats showed highly significant increase in serum ALT, AST, ALP and GT (p<0.001)compared with control negative group. Administration of vitamin C with almond or walnuts showed increase in serum ALT, AST and ALP levels (p<0.05 & 0.01) compared to control negative group but showed improvement in liver function parameters as the increases in these parameters were much lesser than the group which consumed contaminated nuts. Administration of vitamin C also improved serum GT and showed the value somewhat closer to the control negative group (table2).The effects of consumption of aflatoxins contaminated nuts on renal function were illustrated in table 3. Consumption of almond and walnuts alone showed significant increase in serum total bilirubin, creatinine, urea, and uric acid (p<0.05, 0.01&0.001). Consumption of almond with vitamin C showed improvement of renal function as the value of serum total bilirubin was increased (p<0.05) as compared to control negative group and decreased in comparison to consumption of almond only. In the same time, serum creatinine, urea, and uric acid were within normal values in control negative group. Consumption of walnuts in combination with vitamin C showed significant increase in serum total bilirubin, creatinine and urea (p<0.05 & 0.01) as compared to control negative group but these values were lesser when compared with contaminated group. Consumption of almond and walnuts alone showed significant increase in serum cholesterol, TG and LDL-c at p<0.01&0.001 and lower value of serum HDL-c at p<0.05&0.001, respectively, when compared to control negative group. Consumption of almond with vitamin C showed improvement of lipids fractionation as the values of serum cholesterol, TG and HDL-c were within the values of control negative group while LDL-c value was increased at p<0.01 compared to control negative group but decreased compared to group which consumed almond only. Consumption of walnuts in combination with vitamin C showed significant increase in serum cholesterol, TG and LDL-c at p<0.05&0.01 and normal HDL-c compared to control negative group (table3). Consumption of almond and walnuts alone showed significant increase in liver cholesterol at p<0.05&0.01 and significant decrease in liver TG and glycogen at p<0.05, 0.01&0.001 while consumption of walnuts showed significant increase in liver total lipids at p<0.01 when compared to control negative group. Consumption of almond and walnuts with vitamin C total lipid, triglyceride and glycogen were within normal range (table 4).
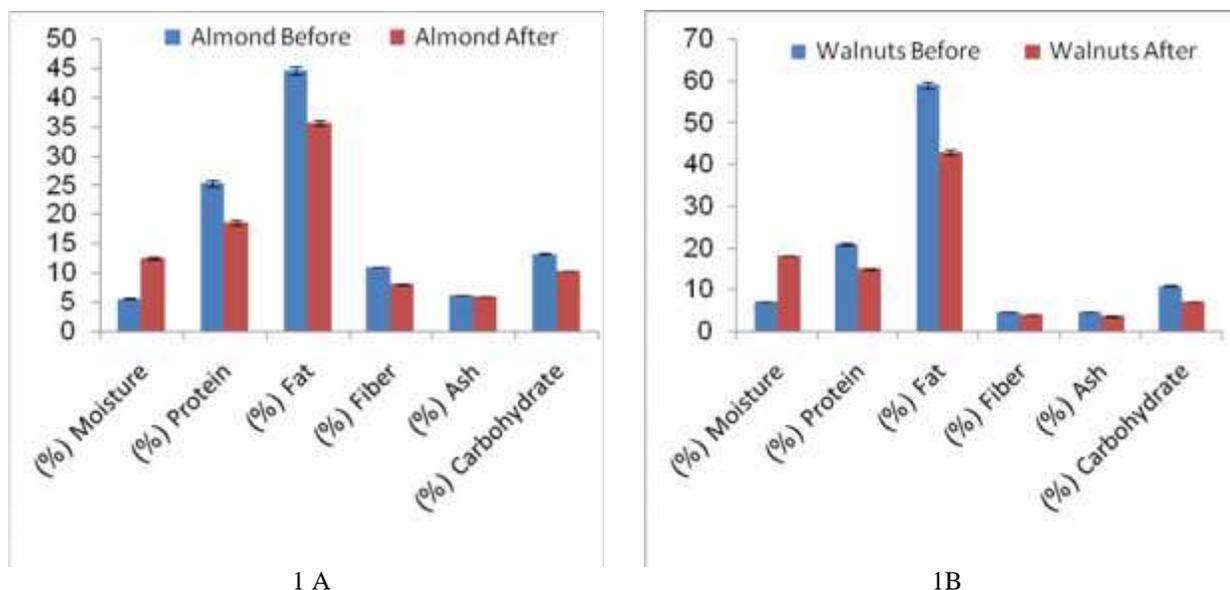
 1 A

 1B

Fig. 1 Proximate composition of almond (Fig 1 A) and walnut (Fig 1 B) before and after contamination.
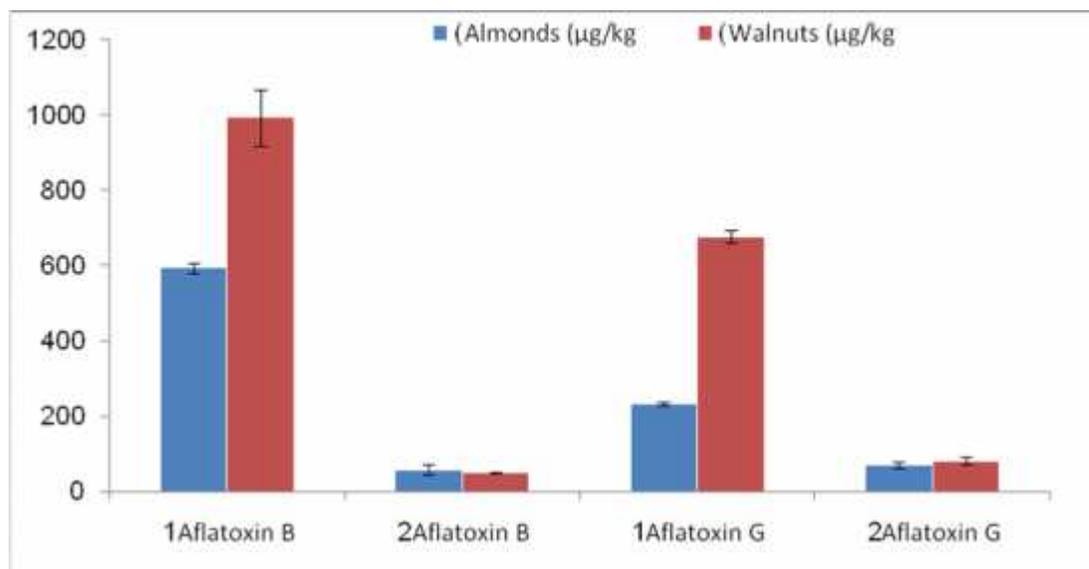

Fig.2. Aflatoxins (B1,B2,G1andG2) levels in almonds and walnuts.

Table 1. Mean values± SD of body weight gain, food intake and FER of the experimental rat groups.

Groups variables	Initial weight	Final weight	Weight gain (g)	Food intake (g/w)	FER
Control negative	180.26±4.46[a]	253.017±22.29[ab]	73.38±7.1[ab]	19.81±1.44[a]	0.087±2.12[c]
Almonds	181.26±4.57a	260.14±24.72a	79.52±8.59a	20.35±1.51a	0.091±0.032[*]
Walnut	182.75±3.62a	267.07±27.65a	84.92±8.9a	19.98±1.38a	0.099±0.25[**]
Almond with vitamin C	180.5±3.41[a]	255.38±26.82[a]	75.49±8.6[a]	20.69±1.38[a]	0.083±1.01[c]
Walnuts with vitamin C	181.28±5.44[a]	263.92±21.75[a]	80.26±9.52[a]	20.23±1.51[a]	0.093±6.81[b*]

Significant with control group [*] $P<0.05$ [**] $P<0.01$ [***] $P<0.001$. Mean values in each column having different letter (a, b, c, d) are significant. WG-weight gain, WG %-weight gain%, FI-food intake, FER- food efficiency ratio, PI- Protein intake and PER-Protein efficiency ratio

Table 2. Mean values ± SD of serum AST, ALT, ALP and   GT (µ /ml) of the   Experimental   rat groups.

Groups Variables	AST	ALT	ALP	GT
Control negative	33.99±3.65^d	27.17±3.40^c	43.09±5.73^c	6.5±0.72^c
Almond	70.63±6.78^{b***}	56.9±7.44^{ab***}	87.358±8.54^{b***}	15.44±1.8^{a***}
Walnuts	93.09±8.64^{a***}	73.24.±6.86^{a***}	120.074±12.72^{a***}	17.87±2.64^{a***}
Almond with vitamin C	42.63±4.43^{c*}	32.41 ±3.69^{d**}	54.89±6.21^{d**}	7.71±1.0^{bc}
Walnuts with vitamin C	52.06±5.50^{c*}	40.63±4.23^{c**}	65.74±6.68^{c**}	8.58±1.28^{bc}

Significant with control group * P< 0.05 ** P<0.01 *** P<0.001. Mean values in each   Column having different letter (a, b, c, d) are significant. (AST)-- Serum Aspartate, (ALT) -- Alanine amino Transferase, (ALP) -- Alkaline Phosphates and   GT gamma Glutamyle Transferase enzymes

Table 3. Mean values ±SD of serum total bilirubin,, creatinine, urea, uric acid , cholesterol, TG,HDL-c and LDL-c of the experimental rat groups.

Parameters	Control negative	Almond	Walnuts	Almond withvitamin C	Walnuts with vitamin C
Total bilirubin	0.688±0.076^d	1.345±0.16^{a***}	1.83±0.36^{a***}	1.01±0.07$^{c*.}$	1.07±0.36^{bc**}
Creatinine	0.582±0.01^d	1.05±0.04^{ab*}	1.16±0.12^{a***}	0.59±0.05^d	0.92±0.08^{c*}
Urea	35.67±3.07^c	51.16±5.29^{ab**}	54.81±6.87^{a***}	35.94±4.3^c	42.45±5.68^{b**}
Uric acid	3.82±0.6^b	4.77±0.82^{a**}	5.21±0.68^{a**}	3.79±0.41^b	4.09±0.7^b
Cholesterol	82.73±8.82c	116.69±12.92^{ab*}	150.25±16.61a**	90.09±10.12^{bc}	100.82±10.38^{b*}
TG	58.58±5.41c	66.82±6.57b**	85.60±9.22a***	60.14±5.52bc	67.97±7.65ab
HDL-c	37.17±3.99^a	27.64±3.44b*	22.104±3.41c*	30.64±4.28^a	30.83±4.22^{ab}
LDL-c	33.85±4.62^d	75.66±9.00^{b***}	111.01±0.93^{a***}	47.04±5.47^{c**}	52.17±5.99^{c**}

Significant with control group * P<0.05 **   P<0.01 *** P<0.001.Mean values in each column having different letter (a, b, c, d) are significant.TG-triglycerides, HDL- high density lipoprotein, LDL- low density lipoprotein.

Table 4. The Mean values ± SD of liver cholesterol, total lipid, triglyceride and glycogen (mg/g) of the experimental rat groups.

Groups Variables	Cholesterol	Total Lipids	Triglyceride	Glycogen
Control negative	4.11±0.68c	36.8±4.36^b	3.54±0.57^a	5.81±1.13^a
Almond	4.94±.92^{ab*}	41.90±6.61^{ab}	3.16±0.65^{b*}	5.16±1.08^{bc**}
Walnuts	5.56±0.38a**	47.29±7.3a**	2.3±0.27bc*	3.94±0.96^{c***}
Almond with vitamin C	4.03±o.10^c	39.21±5.92^b	3.77±0.6^a	6.15±1.22^a
Walnuts with vitamin C	4.35±0.93^{bc}	40.34±7.2^{ab}	3.20±0.37^a	5.5±1.45^a

Significant with control group * P<0.05   ** P<0.01   *** P<0.001. Mean values in each raw having different letter (a, b, c, d) are significant

DISCUSSION

The results of chemical composition suggest that nuts are the edible seed presenting a high content of protein, fat and dietary fibers (mainly insoluble dietary fiber). The protein and lipid contents and the energy value of the almond are similar to those reported in the literature (Fernandez *et al*., 2010). The high protein and oil of the walnut kernels and high levels of mineral elements indicates their potential use in human, animal and poultry feed supplements. The results in Fig 1 A and 1B showed highly significant decrease in proteins and fats with increase in moisture which is in agreement with (Zubair *et al*., 2011) who found a decrease in protein content with increase in aflatoxin concentration especially B1 and G2, while the aflatoxin B1, B2 and G1 showed negative correlation (r < -0.05) with a decrease in fat content. (Iqbal *et al*., 2004) also reported similar data.

The walnuts were infested with various molds and it was also observed that the Aspergillus species (Aspergillus flavus) were toxigenic which can grow and produces aflatoxin in walnuts. The Aspergillus growth and its toxin producing capacity were dependent on nuts composition and certain nutrients enhanced the toxin production. The present study is in accordance with the result reported by (Saleemullah *et al*., 2006) and Savage (2001). Fungal growth on nuts not only produce mycotoxins product but also can decrease the quality and nutritive value of nuts Singh and Shukla (2008).The obtained results in (Figure 2) were in agreement with

(Leong *et al*., 2010) who reported that nut and nutty products in Malaysia were contaminated with aflatoxin ranging in level from16.6 µg/kg up to 711µg/kg. Wang and Liu 2006 reported that the average level of contamination of Chinese peanut was 80.3µg/kg and the highest level was 437µg/kg. Nuts are food samples which are susceptible for this contamination because of their composition and storage conditions. Peanuts, cotton seed, sunflower seed, tree nuts, pistachio, peanut butter, maize flour, pea, cereals, corn, meats, spices, dairy products are major commodities affected by aflatoxin. Aflatoxin is very dangerous for health because of its hepatotoxic, teratogenic, immunosuppressive and mutagenic nature. B1 (AFB1) aflatoxins are regulated in more than 75 countries. Currently the worldwide range of limits for (AFB1) and total aflatoxins are 1–20 ng and 0–35ng respectively, while the European Union (EU) has set a maximum level at 8 µg/kg in samples (Wesolek and Roudot, 2014; FAO, 2004).The effects of consumption of aflatoxins contaminated nuts on body weight , weight gain and FER were illustrated in (table 1) which shows that the consumption of aflatoxin contaminated almonds and walnuts alone or in combination with vitamin C showed significant increase in FER at $p<0.05$ and 0.01 compared to control negative group. These results are in agree with (Alpsoy *et al*., 2009) who reported that vitamin C is a strong reducing agent and as an antioxidant is involved in prevention of the damaging effects of free radicals. Vitamin C is annon- enzymatic antioxidant, co-factor and also increases the gastrointestinal absorption of non-haem irony)**.**Vitamin C or ascorbic acid is an essential vitamin to humans and other mammals that lack the ability to synthesize this vitamin, as they are deficient in the enzyme L-gulonolactone oxidase, involved in the biosynthesis of vitamin C via the glucuronic acid pathway (Kim *et al*., 2014). The biological function of vitamin c is based on its ability to donate electrons. In mammalian cells, vitamin C serves as cofactor for reactions that require reduced iron and /or copper metal C o-enzymes. Another important indirect function of vitamin C is its ability to regenerate other biologically important antioxidants such as I glutathione and vitamin E into their reduced state (Singh *et al*., 2011).In study on effect of vitamin C on aflatoxin, it was observed that values of AFB_1-DNA adduct formation were 9.38±0.41 ng/ml in the AFB_1 treated groups, but the amount formation decreased more significantly to 5.28±0.32 ng/ml in the groups treated with AFB1 and vitamin C ($p<0.01$). Immunohistochemistry revealed that the accumulation of the AFB_1 is not observed in the normal liver tissue (G1).The result in (table 2) is in agreement with earlier studies which revealed significant reductions in enzymatic and non-enzymatic antioxidants in AFB1 fed rat liver. Almond kernels may contain natural antioxidants or enzyme inhibitors which inhibited LOX activity in the homogenates of almonds Abdel-Wahhab

and Aly (2003).Possible mechanism behind the higher liver enzymes could be through regenerative changes and hypo function of liver from aflatoxins. (Meki *et al*., 2004 ; Rastogi *et al*., 2001) recorded that aflatoxins are mutagenic, hepatotoxic and hepatocarcinogenice both for humans and animals and cause oxidative stress even in small amounts. Oxidative damage mainly leads to dysfunction of cellular components such as enzymes, nucleic acids, membranes and proteins. (Abdel-Wahhab *et al*., 2006) reported that the increased activities of ALT, AST and ALP have been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and they are released into plasma as result of autolytic breakdown or cellular necrosis into circulation after cellular damage. On the other side, the mechanism by which vitamin C decrease the hepatotoxicity is by decreasing lipid peroxidation and altering antioxidant defence system or by denoting electrons to free radicals and quenching their reactivity (El-Gendy *et al*.,2010; Ahmad and Al-Jawary, 2012).

        The present study demonstrates that aflatoxin has harmful and stressful influence on the hepatic and renal tissue. The increased levels of uric acid may indicate protein catabolism and/or kidney dysfunction (Kang *et al*., 2005) These results (table3) clearly show that aflatoxicosis significantly increased creatinine, bilirubin, urea nitrogen, alkaline phosphatase and transaminase concentrations (Abdel- Wahhab *et al*., 2006). Similarly, Choudhary and Verma (2005) studied aflatoxicosis in mice and found increased lipid peroxidation and decreased enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase and non-enzymatic antioxidants such as glutathione and ascorbic acid; soaflatoxins are potent nephro- toxic compound leading to severe degenerative renal damage. Recent evidence shows that vitamin C prevents hepatic glutathione depletion in chemical induced hepatotoxicity in mice, in which glutathione worked as intracellular free radical scavengers and protected cells against radical mediated lipid per-oxidation (Cuddihy *et al*., 2008). Another interesting result from the present study was the effect of aflatoxins on lipid fractionations. Rats fed aflatoxins contaminated diet showed a significant increase in total cholesterol, triglycerides and LDL-c accompanied with a significant decrease in HDL-c (Hassan *et al*., 2013). This elevation in serum cholesterol, TG and LDL-c are probably associated with biliary obstruction and acute hepatic injury (El-Nekeety *et al*., 2011). Vitamin C increase HDL-c level and may also lower total cholesterol in the blood, thus reducing the risk of cardiovascular disease. The effect of consumption of vitamin C was in agreement with (Rafighi *et al.,* 2011). They indicated that the use of antioxidants reduces oxidative stress.Vitamin C increase HDL-c level and may also lower total cholesterol in the blood, thus reducing the risk of cardiovascular disease. It

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is well documented that the cholesterol is the principal sterol found in all tissues and body fluids of animals and human beings. In addition to dietary sources, cholesterol can be biosynthesized actively and gets distributed all over the body through blood.The obtained result(table4) was agreed with (Jha *et al*., 2012) who found a significant rise in cholesterol content which might be due to fatty infiltration and degeneration of hepatocytes during aflatoxicosis as toxin is fat soluble. Once brought to the liver through hepatic portal system, fat present in the liver cells might dissolve toxin and retain it. Aflatoxins characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism (Souza *et al*., 1999; Farombi *et al*., 2005). Long term vitamin C supplementation causes reduction in serum total cholesterol and LDL-c significantly but it has no statistically significant lowering effect as far as HDL-c, VLDL-c and triglycerides are concerned in normal human subjects. Therefore prolonged vitamin C supplementation which is also an important constituent of the antioxidant system may help in keeping lipid profile in normal limits and may contribute in delaying the process of atherosclerosis in healthy individuals (Gaur and Dixit, 2011).

Conclusion: Good supervision is necessary for the production and storage of nuts in order to control the aflatoxin at its lowest possible level. It is recommended to administrate vitamin C as antioxidant to lower side effects of aflatoxicosis. The safety of food must be assured by a preventive approach based on the application of a Hazard Analysis Critical Control Point (HACCP) at all stages of food chain.

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