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Effect of poor storage of peanuts and almonds on chemical composition, aflatoxin and biological changes of experimental rats

تأثير سوء تخزين الفول السوداني واللوز على التركيب الكيميائي والأفلاتوكسين والتغيرات البيولوجية لفئران التجارب

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Abstract

Poor storage and Aflatoxin contamination of nuts is an increasing concern to the consumer's health so the current study was undertaken to analyses the effect of poor storage on chemical composition, aflatoxin and experimental rats in nuts (peanut and almond). Peanut and almond were store 18 month and The effect of poor storage nuts was investigated on experimental rats Thirty white albino rats were randomly classified into five groups: (1) control negative, (2&3) group will be fed on100g fresh and storage peanut /kg diet, respectively. (4&5) group will be fed on 100g fresh and storage almonds /kg diet, respectively. The results showed the find fungi in peanuts and almonds after poor storage, which led to the appearance aflatoxin B1 and B2 were higher in peanut about almond after 18months storage. The results suggested that protein and fat decreased significantly, while highly

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significant increase was observed in moisture content.. Showed in final, body weight reduce and food intake as compared to control negative group. Almond and peanut the higher level serum ALT, AST and ALP in the store group unlike fresh group. showed significant increase in serum Uric acid, Urea nitrogen and creatinine compared to control negative group.

الملخص:

سوء التخزين وتلوث المكسرات بالأفلاتوكسين مصدر قلق متزايد لصحة المستهلك لما لها من تأثير ضار لذلك أجريت هذه الدراسة الحالية لتحليل تأثير التخزين السيئ للمكسرات (الفول السوداني واللوز) على التركيب الكيميائي والأفلاتوكسين وفئران التجارب. تم تخزين الفول السوداني واللوز لمدة ١٨ شهرً وتمت دراسة تأثير سوء التخزين على فئران التجارب وتم تصنيف ثلاثين من القئران البيضاء بشكل عشوائي إلى خمس مجموعات: (١) سلبية ، (٢ و ٣) ستتغذى على ١٠٠ جرام من الفول السوداني الطازج والمخزن / كجم على التوالي. مجموعة (٤ و ٥) ستتغذى على ١٠٠ جرام لوز طازج ومخزن / كجم على التوالي. وأظهرت النتائج ظهور فطريات في الفول السوداني واللوز بعد التخزين السئ مما ادي الي ظهور سموم الأفلاتوكسين التى كانت اعلى فى القول السودانى عن اللوز . واظهرت النتائج ايضا انخفاض في البروتين والدهون والكربوهيدرات ، بينما لوحظ ارتفاع في محتوى الرطوبة. وظهر في نهاية التجربه انخفاض فى وزن الجسم مقارنة بمجموعة التحكم السلبية. وارتفعت نتائج وظائف الكبد على عكس مجموعة الطازجة.

INTRODUCTION

The peanut or groundnut (Arachis hypogaea) is a species in the legume family, Fabaceae. It is one of the major oilseed crops of the world. It is world's fourth most important source of edible vegetable oil and the third most important source of vegetable protein feed meal. Peanuts can be consumed as raw, roasted or mixed with other foods or in different processed forms. Recently, peanuts have gained much attention as functional food. In the United States, the consumption of peanuts is greater than all the other nuts combined.(Attar AML2010).

Recent research on peanuts and nuts in general has found anti-oxidants and other chemicals that may provide health benefits. Also, peanuts are a significant source of resveratol, a chemical studied for potential anti-aging, anti-cancer and anti-inflammatory influences. It has lipid lowering effects and also decreases the body weight. (Karanth J, Jeevaratnam K2005, Anonymous 2001) . Peanuts (Arachis hypogaea L.) are a valuable source of protein and fats for humans and livestock. However, they are prone to various types of deterioration during storage which renders them unsuitable for consumption (Bulaong and Dharmaputra 2002) and trade, resulting in large economic losses (Williams 2008).

Almonds provide important nutrients such as vitamin E, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), arginine, and magnesium. Almonds also contain considerable amounts of potential prebiotic indigestible carbohydrates (Bolling et al., 2010). 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 (Manish, 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)

Aflatoxin is a potent human carcinogen It is a naturally occurring toxic metabolite produced by certain fungi (Aspergillus flavus),al mold found on food products' such as corn and peanuts butter (Felicia, 2004). Numerous studies have linked aflatoxins to various diseases, such as cancer of liver and hepatitis B and C. High levels of aflatoxin were detected in children with kwashiorkor (childhood malnutrition from protein insufficiency) in the Sudan, Durban, South Africa and Nigeria. In Gambia, 93% of sampled children (6-9 years old) were tested and found to be positive for aflatoxin albumin adducts]. [Ephrem Guchi* 2015]. For example, aflatoxinB1 (AFB1) is classified as a Group I carcinogen by the International Agency for Research on Cancer for its acute toxicity on liver and kidney in both human and animals (Zhao Penga, et all 2018).

Material and Methods

Materials:

1- Peanuts and almonds will be obtained from local market, Cairo, Egypt.

2- Casein, all vitamin, minerals, cellulose and choline chloride will be obtained from Elgamhoria Company, Cairo, Egypt.

3- Normal male albino rats (30) of Sprague Dawley Strain weighted 100 ± 5 g. Will be obtained from the laboratory animal colony, Helwan, Cairo, Egypt.

Methods:

Storage of peanut and almonds: Peanuts and almonds will be storage at room temperature for 18 month.

Chemical composition:

Moisture, crude protein, crude fat, ash, crude fiber and fatty acid composition of peanut and almonds will be determined before and after storage according to A.O.A.C (2000), while total carbohydrates content will be calculated by according to the methods of the A.O.A.C. [1995],.

Peroxide value: Peroxide value will be determined in oil, which extracted from peanut and almonds before and after storage.

Fungi

Seed of peanuts (Arachis hypogaea L.) and almond (Prunes Amygdalus L) collected from nuts. local market, Cairo, Egypt. Was examined for their contamination with fungi at plant pathology research Institute (ARC) Giza, Egypt.

Aflatoxin

Aflatoxin content in peanut and almonds will be determined before and after the storage period according to the method described by Park et al. (1990).

Biological part :

Male albino rats sperague Dawely strain weighting (100 ± 10 g) will be housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation. After this period, the rats will be divided into five groups. The first group (6 rats) will be fed on basal diet. The basal diet in the preliminary experiment consists of 20% casein (protein > 80%), .Sun flower oil 5%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the remainder is corn starch (Reeves et al., 1993). Vitamin composition of diets prepared according to (A.O.A.C., 1975).

The second and third groups will be fed on basal diets containing 100g fresh and stored peanut /kg diet, respectively.

The fourth and fifth groups will be fed on basal diets containing 100g fresh and stored almonds /kg diet, respectively.

At the end of the experimental period; rats will be fasted overnight before sacrifice, blood will be collected from each rat, and centrifuged to obtain the serum. Liver and kidney will be removed from each rat and weighted.

B- Laboratory Analysis

-*Biochemical analysis of serum:

Liver function was determined as follows:-

a) - Serum alanine amino transferase and aspartate amino transferase enzymes activity (ALT & AST) were performed according to the method of Reitman and Frankel (1957).

B) - Kinetic determination of alkaline phosphates (ALP) in serum or plasma samples was performed according to [Devi et al. (2000) and Draper & Handley (1990)].

Results and discussion

Data in table (1 and 2) showed that, 7 fungal species were found to be associated with peanut. In the first stage (6 month) appear 3 fungal A. Flavus, A.Niger and Penicilium sp. (12.3 & 16.4 & 7.2%) then increase in stage two (12 month) (14 & 20 & 10) frequently It also appears at this stage Rhizopus spp with 6.7%, and at the end of the experiment, it was found 7 fungal with peanut, the Aspergillus sp were the most prevalent and constituted 55.0% in which (A.Flavus constituted 20.0 % and A.Niger constituted 30.7%) while the Penicilium sp. Ranked the third with (13.0%) then Rhizopus sp the the fourth with (10%), then the following Alternaria with (8%), the less frequently fungi were Fusaviom sp and Epicocum sp (6.7%) respectively.

While the results not appear many difference between almond and peanut. Table (5) revealed that, storage almond for 6 months appeared Aspergillus niger and A.Flavus, (8.7 & 8.4%) and after 12 month storage increased Aspergillus niger and A.Flavus to (14 & 11.2%). The results in this Table appeared percentages of Penicilium sp. and Alternaria spp (5.2 & 2.3%) after 12 month, at the end of the experimental period the obtained results showed increase in Aspergillus niger, A.Flavus and Penicilium sp increased to (25, 17 and 9%) followed by Alternaria spp (6.7%), but were not find fungi. (Fusaviom sp , sp, Epicocum sp). In this respect, Shank et al .,(1972) and Singh and Shukla (2008). Reported that, Fungal growth on nuts not only produce mycotoxins product but also can decrease the quality and nutritive value of nuts

Table (1) Frequency (%) of fungi isolated from peanut seed

Isolated fungi	Control	6 month	12 month	18 month
A.Flavus	**2.4**	**12.3**	**14.0**	**20.0**
A.Niger	**6.8**	**16.4**	**20.0**	**30.7**
Fusarium sp.	**0.0**	**0.0**	**0.0**	**6.7**
Alternaria spp	**0.0**	**0.0**	**0.0**	**8.0**
Penicilium sp.	**0.0**	**7.2**	**10.0**	**13.0**
Rhizopus spp	**0.0**	**0.0**	**6.7**	**10.0**
Epicocum spp	**0.0**	**0.0**	**0.0**	**6.7**

Table (٢) Frequency (%) of fungi isolated from almond seed

Isolated fungi	Control	6 month	12 month	18 month
A.Flavus	**1.6**	**8.4**	**11.2**	**17.0**
A.Niger	**1.2**	**8.7**	**14.0**	**25.0**
Fusarium sp.	**0.0**	**0.0**	**0.0**	**0.0**
Alternaria spp	**0.0**	**0.0**	**2.3**	**6.7**
Penicilium sp.	**0.0**	**0.0**	**5.2**	**9.0**
Rhizopus spp	**0.0**	**0.0**	**0.0**	**0.0**
Epicocum spp	**0.0**	**0.0**	**0.0**	**0.0**

Aflatoxins ratio in poor storage stages in peanuts and almond

Data in table (٣) showed that, concentrations of aflatoxins in peanut and almond samples after storage for (6, 12 and 18 month). High concentrations of (**B1, B2, G1 and G2)** appear in the last stage of storage (after storage for 18 month)

In the second stage of storage (six months) did not show any toxins, while it began to appear in the next stage (storage 12 month) in B1 and B2, then the toxins increased in the last stage of storage, the percentage of toxins in B2 is greater than B1.

The table also showed that, no toxins from G1 and G2 were detected in the nuts storage stages. The results showed that the percentage of aflatoxin in peanuts is more than in almonds. **(Ozilgen & Ozdemir, 2001; Schatzki & Ong, 2001)**.

As aflatoxins are highly toxic and carcinogenic substances, their maximum levels have been regulated. The amount of those substances are the sum of the B1,B 2,G 1 and G2 aflatoxins, the highest permitted levels being 4mg kg 1 for groundnuts, nuts and dried fruit and processed products

thereof, which are intended for direct human consumption and 10mg kg1 as an ingredient in foodstuffs and for nuts and dried fruit to be subjected to sorting, or other physical treatments, before human consumption or for use as an ingredient in foodstuffs, respectively (**Commission Regulation (EC) No. 466/2001, 2001).**

Hell et al., (2008) reported that, aflatoxin production by Aspergillus spp. can increase tenfold in a three-day period, if harvested grains are stored with a high moisture content

The Codex Alimentarius Commisssion (CAC) Joint Food and Agricultural Organization of the United Nations and the World Health Organization food standards program adopted a level of 15 mg/kg for AFT for unprocessed peanuts and 10 mg/kg for ready-to-eat tree nuts (**Ding et al., 2012).**

For peanuts, nuts, dried fruits and cereals, the maximum level of 2 ng/g for B1 and 4 ng/g for total aflatoxins have been set by the European Commission (**Afsah-Hejri et al., 2011).** The European Union (EU) has set a maximum level at 8 µg/kg in samples (**FAO, 2004 and Wesolek, and Roudot, 2014)**

Table (3) Concentrations of aflatoxins (µg/kg) in peanut samples

	Peanut (ppm)					Almond (ppm)				
	B1	B2	G1	G2	total	B1	B2	G1	G2	Total
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6 month	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12 month	5	4	0.0	0.0	9	2.7	2.3	0.0	0.0	4.9
18 month	9	10	0.0	0.0	19	4.8	5.1	0.0	0.0	9.9

Chemical Composition of Almond and Peanut Before and After Storage.

Chemical composition of fresh peanut including protein, fat, ash, Moisture, fiber and carbohydrate were (26.4, 44.9, 2.4, 4.6, 2.9 and 18.8 gm/100g), respectively while peanut storage for 18 month led to decreased amount of protein, fat, ash, fiber and carbohydrate (22.6, 41.8, 2.1, 2.8 and 15.9 gm/100g), the storage period for 18 month increased the amount of moisture content from (4.6 to 14.8g/100g).

Poor storage of peanuts and almond increased the mean values of peroxide by about 99.123% and 93.494% respectively, than that of fresh nuts.

Chemical composition of fresh almond including protein, fat, ash, moisture, fiber and carbohydrate were (17.6, 55.8, 2.7, 4.6, 2.5 and 16.8 gm/100g), respectively. Storage almond for 18 month decreased the amount of all nutrients, except moisture which increased with storage. In this respect, our results in the amount of protein, lipid and the energy value of almonds are agreement with those reported by (**Fernandes et al., 2010).**

Storage conditions of peanuts play a vital role in their quality, owing to their high oil content, that deteriorates depending on conditions under which the nuts are stored. After harvest, it is recommended that peanut kernels should be dried to safe moisture levels ≤ 10% (**Rahmianna and Yusnawan 2007, WHO/FAO 2012).**

The results in table (٣,4) showed B1 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.

Table (٤) Chemical Composition (µg/kg) of Almond and Peanut Before and After Storage (g/100g).

Types / Nutrients	Peanuts F	Peanuts S	Almonds F	Almonds S
Protein	26.4	22.6	17.6	14.27
Fat	44.9	41.8	55.8	53.6
Ash	2.4	2.1	2.7	2.5
Moisture	4.6	14.8	4.6	13.83
Fibre	2.9	2.8	2.5	2.3
CHO	18.8	15.9	16.8	13.5
Calories	585	530.2	640	593.48
Peroxide value	17.12	34.09	16.91	32.72

Effect poor storage of peanuts and almonds on fatty acids composition

Data in table (٥) showed the effect of poor storage of peanuts and almonds on the fatty acids composition of their oils. After poor storage of peanuts, changed in total Saturated fatty acid (SFA), Mono unsaturated Fatty acid (MUFA) and poly unsaturated Fatty acid (PUFA) occurred. Total SFA reduced in peanuts which stored poorly than that of fresh (16.33% vs. 18.1%), respectively. Total Mono-unsaturated fatty acids decreased in peanuts after poor storage than that of fresh (50.63% vs. 57.41%), respectively. Total PUFA decreased in peanuts after storage poorly, as compared to the fresh peanuts (24.59% vs. 35.01%), respectively. The same trend was observed in almonds.

Reed et al. (2002) reported that, the presence of the high amount of oil contained in peanut kernels, their quality can deteriorate quickly due to lipid oxidation depending on the presence of oxygen, light, moisture, and high temperatures. While, **(Pomeranz, 1992 and Bulaong & Dharmaputra**

2002) reported also, storage fungi can change fat quality of peanuts by hydrolytic enzymes producing free fatty acids and glycerol**,** which lead to lower quality or rejection of foodstuffs.

Table (٥) Concentrations of fatty acid (µg/kg) in peanut and almond samples before and after storage.

Nuts / Fatty acid profile		Peanut (F)	Peanut (S)	Almond (F)	Almond (S)
Saturated fatty acid (SFA)	Myristic acid C14:0	0.02	0.02	0.02	0.02
	Palmitic acid C16:0	9.49	8.67	5.41	5.64
	Margaric acid C17:0	0.05	0.03	0.03	0.03
	Stearic acid C18:0	3.05	2.86	1.16	1.32
	Arachidic acid C20: 0	1.49	1.23	0.09	0.06
	Behenic acid C21:0	2.6	2.19	0.14	0.06
	Tricosanoic acid C23:0	0.03	0.10	0.00	0.00
	Lignoceric acid C22:0	1.37	1.23	0.06	0.05
	Total SFA	18.1	16.33	6.91	7.18
Mono unsaturated Fatty acid (MUFA)	Palmitoleic acid C16 :1	0.04	0.03	0.05	0.39
	Oleic acid C18:1	56.38	49.67	67.18	65.10
	Eicosenoic acid C20:1	0.99	0.93	0.09	0.06
	Total MUFA	57.41	50.63	67.32	65.55
Poly unsaturated Fatty acid PUFA	Linoleic acid C18:2	34.98	24.54	27.18	25.19
	Linolenic C18:3	0.03	0.05	0.03	0.02
	Linoelaidic acid C18:2			0.08	0.06
	Total PUFA	35.01	24.59	27.29	25.27

Effect of poor storage of peanuts and almonds on feed intake and BWG% of healthy rats.

The effect of fresh and poor storage of peanuts and almonds on feed intake (g/day/each rat) and body weight gain% of healthy rats presented in Table (٦) and illustrated in Fig. (١ and ٢).

Feed intake (g/day/each rat):

The mean value ± SD of feed intake of the control group (healthy rats) was 13.600 ± 0.547 (g/day/each rat). Results of feeding rats on diet containing 10 % fresh (peanuts or almonds), showed No -significant changes in feed intake, as compared to the control group fed on basal diet. While, feeding healthy rats on diet containing 10 % (peanuts or almonds) which were stored poorly led to significant decreased in the mean values of feed intake, as compared to control group and other treated groups.

Table (٦): Effect of poor storage of peanuts and almonds on food intake and BWG% of healthy rats.

Parameters / Groups		Food intake (g/day/each rat)	Body weight gain% (BWG%)
Rats fed on	Basal diet (Control group)	13.600 [a] ± 0.547	34.882 [a] ± 1.718
	Diet containing 10% fresh peanut /kg diet	13.200 [a] ± 0.447	31.134 [b] ± 2.489
	Diet containing 10% stored peanut /kg diet	12.200 [b] ± 0.836	25.134 [c] ± 1.010
	Diet containing 10% fresh almonds /kg diet	13.400 [a] ± 0.547	34.452 [a] ± 1.922
	Diet containing 10% stored almonds /kg diet	12.200 [b] ± 0.731	24.854 [c] ± 2.785

Least significant differences at P≤0.05.

Means with the same letter are insignificantly difference.

Body weight gain %:

The effect of fresh and poor storage of peanuts and almonds on body weight gain% (BWG%) of healthy rats presented in the same Table and illustrated in fig. (٤). The results indicated that, feeding rats on basal diet (control group) recorded weight gain of about (34.882 %). BWG% of rats which fed on diet containing 10% fresh almonds showed non-significant changes, as compared to control group, while BWG% of rats which treated with 10% peanuts recorded significant decrease $p \leq 0.05$, as compared to control group. On the other hand, poor storage of peanuts and almonds caused significant decrease in BWG% of rats, as compared to control group.

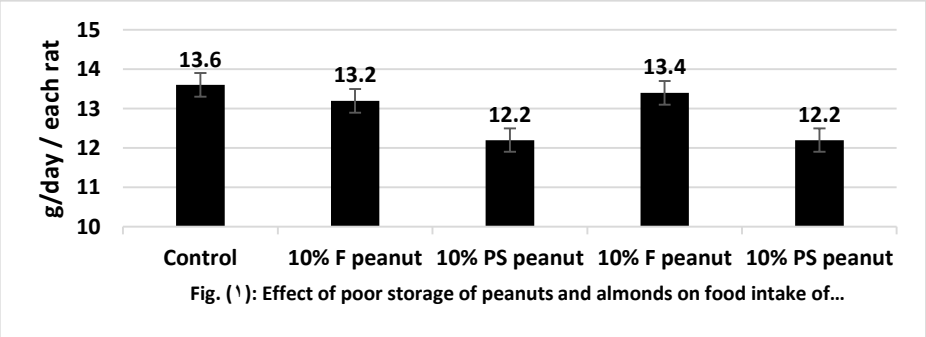

Fig. (١): Effect of poor storage of peanuts and almonds on food intake of…

F: Fresh PS: Poor storage

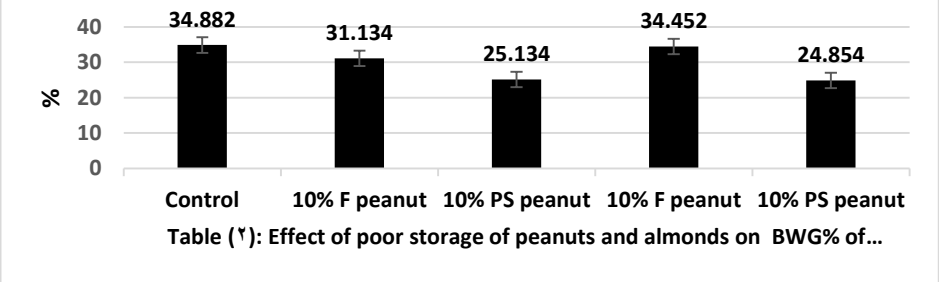

Table (٢): Effect of poor storage of peanuts and almonds on BWG% of…

F: Fresh PS: Poor storage

In this respect, **Sakata et al., (2003)** reported that, textural property of almonds could promote satiety. **(Slavin, 2005)** reported that, almonds are a significant source of fibre, a food constituent with documented satiating properties**.**

The main factors contributing to the less-than predicted effects of nut ingesting on body weight gain are their strong satiety effects, incomplete energy bioaccessability and possible augmentation of resting energy expenditure **(Mattes et al., 2008).** Epidemiologic studies have shown a negative or inverse relation between nut intake and body weight **(Mozaffarian et al., 2011)**. Mechanisms underlying the relation between nut intake and weight are unclear but may be related to alter resting energy expenditure, inefficient absorption of energy from nuts, or increased satiety **(Alper and Mattes 2002 and Hollis and Mattes 2007)**. In addition to epidemiologic evidence, controlled feeding studies advise that nuts do not promote significant weight gain (**Li et al., 2010).**

According to **Penny and Peter (2015) found that,** diet containing 84 g/ day of almonds decreased weight gain by 14% compared with a 9% decrease with an isocaloric, complex carbohydrate control diet wherefore almond reduce body weight. On the other hand, **Bes-Rastrollo and Wedick (2009)** recognized that nuts have a healthy fatty acid profile and nut consumption has been associated with reduced BMI and advocated in weight maintaining diets.

Mattes et al (2008) reported that, final body weight of peanut treated group was significantly lower in comparison to that of control group. Some investigators observed similar type of findings.

Effect of poor storage of peanuts and almonds on liver enzymes of healthy rats

The effect of fresh and poor storage of peanuts and almonds on liver enzymes including (Aspartate Amino Transferees AST, Alanine Amino Transferees ALT and Alkaline phosphatase ALP) of healthy rats presented in Table (15) and illustrated in Fig. (٣ , ٤ and ٥).

Aspartate Amino Transferees AST (u/l):

The mean value ± SD of serum AST enzyme in the control group which fed on basal diet was (33.388 ± 2.558 u/l). Feeding rats on diets containing 10% fresh (peanuts or almonds) caused non-significant difference $p \leq 0.05$ in the mean values of AST enzyme, as compared to control group, on the other hand, treating rats with 10% peanuts or almonds, which were stored poorly induced significant increase $p \leq 0.05$ in serum AST, as compared to control group and other treated groups (Table ٧ and Fig.٦).

Treating rats with 10% peanuts or almonds, which were stored poorly led to significant increase $p \leq 0.05$ in serum AST enzyme, as compared to rats which treated with 10% fresh peanuts and almonds.

Storage peanuts and almonds poorly, increased the mean value of serum AST enzyme by about 33.407% and 69.701% than that of the control, respectively.

Table (٧) : Effect of poor storage of peanuts and almonds on liver enzymes of healthy rats.

Parameters / Groups		AST	ALT	ALP
		U/L		
Rats fed on	Basal diet	33.388 ^{c} ± 2.558	13.094 ^{c} ± 1.286	499.520 ^{c} ± 7.029
	Diet containing 10% fresh peanut /kg diet	32.970 ^{c} ± 1.576	16.582 ^{b} ± 1.250	509.120 ^{c} ± 15.444
	Diet containing 10% stored peanut /kg diet	45.210 ^{b} ± 0.717	32.296 ^{a} ± 3.469	867.340 ^{a} ± 53.014

	Diet containing 10% fresh almonds /kg diet	32.630 [c] ± 2.967	17.836 [b] ± 2.110	516.060 [c] ± 6.804
	Diet containing 10% stored almonds /kg diet	56.660 [a] ± 2.771	35.206 [a] ± 2.167	761.540 [b] ± 81.501

Least significant differences at P≤0.05.
Means with the same letter are insignificantly difference.

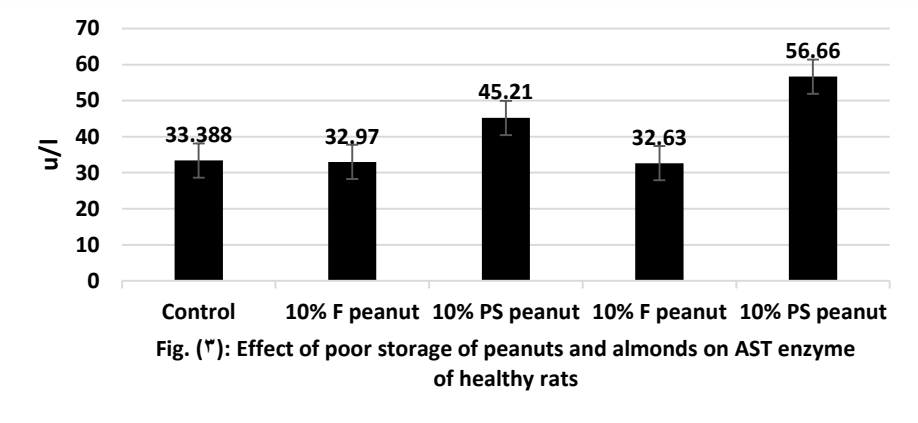

Fig. (٣): Effect of poor storage of peanuts and almonds on AST enzyme of healthy rats

F: Fresh PS: Poor storage

Alanine Amino Transferees ALT (u/l):

The mean value ± SD of serum ALT enzyme in the control group which fed on basal diet was (13.094 ± 1.286 u/l). Feeding rats on diets containing 10% peanuts or almonds (fresh or which were stored poorly) induced significant increase p ≤ 0.05 in the mean values of ALT enzyme, as compared to control group (Table ٧and Fig. ٧).

Data in this Table revealed that, non-significant changes in the mean value of serum ALT enzyme between the groups of rats which were treated with 10% fresh peanuts and almonds. The same trend was observed in the groups which treated with 10% peanuts and almonds, which were stored poorly.

Treating rats with 10% peanuts or almonds, which were stored poorly led to significant increase $p \leq 0.05$ in serum ALT enzyme, as compared control group and the rats which treated with 10% fresh peanuts and almonds.

Storage peanuts and almonds poorly, increased the mean value of serum ALT enzyme by about 146.647% and 168.871% than that of the control, respectively.

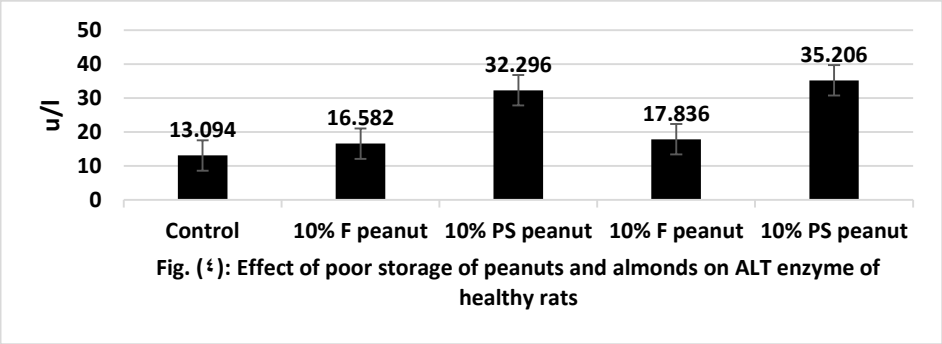

Fig. (٤): Effect of poor storage of peanuts and almonds on ALT enzyme of healthy rats

F: Fresh PS: Poor storage

Alkaline phosphatase ALP (u/l):

The mean value ± SD of serum ALP in the control group which fed on basal diet was (499.520 ± 7.029u/l). Feeding rats on diets containing 10% fresh (peanuts or almonds) caused non-significant difference $p \leq 0.05$ in the mean values of ALP, as compared to control group, on the other hand, treating rats with 10% peanuts or almonds, which were stored poorly induced significant increase $p \leq 0.05$ in this parameter, as compared to control group and other treated groups (Table ٧ and Fig.٨).

Storage peanuts and almonds poorly, increased the mean value of serum ALP by about 73.634% and 52.454% than that of the control, respectively. The highest increase in ALP recorded for the group treated with diet containing 10% stored peanut /kg diet, followed by the group which treated with diet containing 10% stored almonds /kg diet

The results in Table (٧) showed, the effect of fresh and poor storage nuts on liver enzymes including (AST, ALT and ALP) of healthy rats. Findings of this study revealed that, storage nuts (peanuts and almonds) poorly increased the mean values of serum these enzymes, as compared to the groups which were treated with fresh nuts.

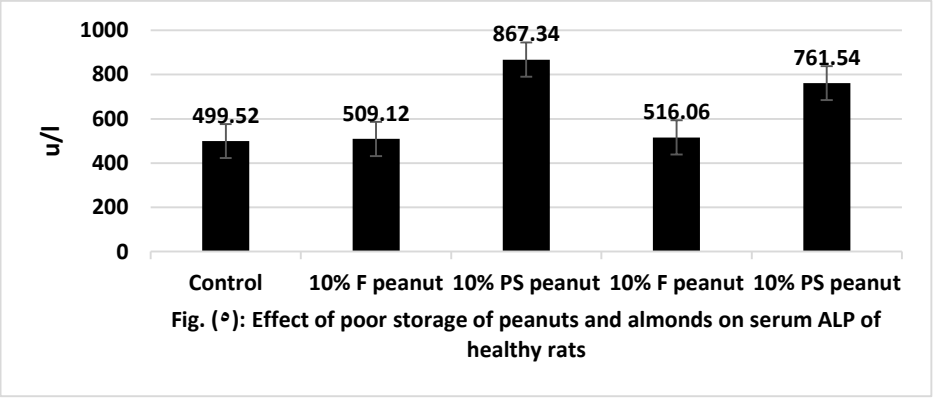

Fig. (٥): Effect of poor storage of peanuts and almonds on serum ALP of healthy rats

F: Fresh PS: Poor storage

In this respect, **(Alasalvar *et al*., 2006, Yang *et al*., 2009 and Choi *et al*., 2002)** reported that, the improvement in liver enzymes in treated diabetic rats with some nuts may be related to the antioxidant properties of these nuts which have scavenge free radicals and thereby may protect cells from oxidative stress. Almonds (Amygdalus communis L.) are a rich source of nutrients and phytochemicals such as vitamin E also polyphenols that is known as antioxidants and had strong free radical scavenging.

Naziroglu et al., (1999) reported that, administered vitamin E has protective effects against CCl_4-induced chronic liver damage and cirrhosis as evidenced by biochemical data and conventional histological examination.

Hepatoprotective activity of the almonds extract against Paracetamol and CCl4 induced hepatitis in rats studied by **Soni et al., (2011)** which reported that, the treatment with the extract of the almonds (150mg/kg and 300mg/kg) brought back the altered levels of the biochemical markers to near normal levels in a dose dependent manner.

potential mechanism behind the higher liver enzymes could be through regenerative changes and hypo function of liver from aflatoxins. **(Meki et al., 2004 and Rastogi et al., 2001)** recorded that aflatoxins are mutagenic, hepatotoxic and hepatocarcinogenice both for animals and humans and cause oxidative stress even in small amounts. Oxidative deterioration mainly leads to dysfunction of cellular components such as nucleic acids,enzymes, membranes and proteins.

Abdel-Wahhab et al., (2006) announce that the increased activities of AST,ALT and ALP have been refer 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 deterioration.

Carcinogens like aflatoxin B1, which generate epoxides, have been found to conjugate readily with GSH liver cells, which are lethally injured by several toxins, exhibit marked alternation in intracellular Ca2+ homeostasis after excessive accumulation of Ca2+. **(Devendran and U. Balasubramanian 2011**

During hepatocellular necrosis, excessive intracellular Ca2+ is known to thrust the metabolism in an unmanageable disorder, which leads to mitochondrial dysfunction inhibition of enzymes and denaturation of structural proteins. (**Devendran and U. Balasubramanian 2011**)

REFERENCES

- **A.O.A.C. (1975):** Official Methods of Analysis of Assoc. of the official Analytical Chemists. 12th Ed. Washington, D. C.
- **A.O.A.C. (1995):** Official Methods of Analysis 16th Ed. Washington, D.C. Association of Official Analytical Chemists.
- **A.O.A.C. (2000):** Official Methods of Analysis of A.O.A.C. International. 17th ed., Gaithersburg Maryland, USA. Chap. 4. pp: 21-39.
- **Abdel-Wahhab, M.A., H. Ahmed and M. Hawaii (2006).**Prevention of aflatoxin B1iniated hepatotoxicity in rat by marine algae extracts. J. Appli Toxic.6:229-238
-**Afsah-Hejri, L., Jinap, S., Arzandeh, S., Mirhosseini, H., (2011).** Optimization of HPLC conditions for quantitative analysis of aflatoxins in contaminated peanut. Food Control, 22, 381-388
- **Alasalvar, C.; Amaral, J.S. and Shahidi, F. (2006)**. Functional lipid characteristics of Turkish Tombul hazelnut (Corylusavellana L.). *J. Agric. Food Chem.,* 54: 10177-10183.
- **Alper, C.M. and Mattes, R.D. (2002).** Effects of chronic peanut consumption on energy balance and hedonics. *Int. J. Obes. Relat. Metab. Disord.;* 26, 1129–1137.
- **Anonymous**, **2001**. Final Report on the Safety Assessment of Peanut (Arachis hypogaea) Oil, Hydrogenated Peanut Oil, Peanut Acid, Peanut Glycerides, and Peanut (Arachis hypogaea) Flour. Int J Toxicol ;20(2):65-77. Applied and Environmental Microbiology, 60(7), 2248-2251.
- **Attar AML**. **(2010)**. Physiological Effects of Some Plant Oils Supplementation on Streptozotocin-Induced Diabetic Rats. Res J Medicine & Med Sci.;5(1):55-71.

- Bes-Rastrollo M, Wedick NM, Martinez-Gonzalez MA, Li TY, (**2009).** Sampson L, Hu FB. Prospective study of nut consumption, long-term weight change, and obesity risk in women. Am J Clin Nutr.;89:1913-9.

- Bolling B.W., McKay D.L., and Blumberg J.B. (2010) The phytochemical composition and antioxidant actions of tree nuts. Asia Pacific Joural of Clinic Nutrition, Vol. 19, p. 117–123.

- Bulaong SSP, Dharmaputra OS (2002) Fungal population, aflatoxin and free fatty acid contents of peanuts packed in different bag types. Biotropia 19:1-25.

- Choi, C.W.; Kim, S.C.; Hwang, S.S.; Choi, B.K.; Ahn, - H.J.; Lee, M.Y.; Park, S.H. and Kim, S.K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci.*, 163: 1161-1168.

- Commission Regulation (EC) (2001). No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L 77, 1–13.

- Devendran and U. Balasubramanian. (2011). Biochemical and histopathological analysis of aflatoxin induced toxicity in liver and kidney of rat. Asian Journal of Plant Science and Research, 2011, 1 (4):61-69.

- Devi, G.S.; Prasad, M. H.; Saraswathi, L.; Raghn, D.; - Rao, D.N.; and Reddy, P.P. (2000): Free radicals antioxidant enzyme and lipid peroxidation in differet types or leukemia. J. Clinical Chemical Act 293.53.

- Ding, X., Peiwu, L., Bai, Y., Zhou, H., (2012). Aflatoxin B1 in post-harvest peanuts and dietary risk in China. Food Control, 23, 143-148.

- **Draper, W.; and Handley, M. (1990):** Indirect determination or oxygen free radicals, methods enzyme. 186,421-431.
- **Ephrem Guchi. (2015)**. Aflatoxin Contamination in Groundnut (Arachis hypogaea L.) Caused by Aspergillus Species in Ethiopia. Journal of Applied & Environmental Microbiology, 2015, Vol. 3, No. 1, 11-19
- **FAO (2004)**.Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper 81
- **Felicia W.U (2004).** Mycotoxin Risk Assessment for the Purpose of Setting International Regulatory Standards .Environ. Sci. Technol. 38: 4049– 4055.
- **Fernandes, C. D., F. B. Jullyana., C. P. Ludmila and N. M. Margareth (2010).** Nutritional Composition and Protein Value of the Baru (DipteryxAlata) almond from the Brazilian savanna. J Sci Food and Agri. 90:1650−1655
- **Food and Agriculture Organization.** (**2012).** Food Balance Sheet. Available online: http://faostat.fao.org/site/614/default.aspx (accessed on 13 November).
- **Hell, K., Fandohan, P., Bandyopadhyay, R., Cardwell, K., Kiewnick, S., Sikora, R., & Cotty, P. (2008).** Pre- and post-harvest management of aflatoxin in maize. In Leslie, J.F. Bandyopadhyay, R., Visconti, A., (Eds) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK. 210-219.
- **Hollis, J. and Mattes, R. (2007).**Effect of chronic consumption of almonds on body weight in healthy humans. *Br. J. Nutr.*; 98:651–6.
- **Karanth J, Jeevaratnam K.** (**2005).** Oxidative Stress and Antioxidant Status in Rat Blood, Liver and Muscle: Effect of

Dietary Lipid, Carnitine and Exercise. Int J Vitam Nutr Res.;75:333-39.

- **Li, L. and Tseng, C. (2010)** . Pistachio nuts reduce trigylcerides and body weight by comparison to refined carbohydrate snack in obese subjects on a 12-week weight loss program. *J. Am. Coll. Nutr*.;29:198–203.

- **Manish, B. (2014)**.Association of nut consumption with total and cause-specific mortality. Indian Heart J. 66: 388–389.

- **Mattes RD, Kris-Etherton PM, Foster GD**, (**2008)**. Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. J Nutr.;138:1741S-5S.

- **Mc, Inory (1954):** Amicro heamatocrit for determining the packed cell and hemoglobin concentration on capillary blood. J.Clin. path., (7): 32.

- **Meki, A. R.,E. E. D. F. Esmail., A.A. Hussein and H.M. Hassanein (2004).** Caspase-3 and heat shock protein-70 in rat liver treated with aflatoxin B1:Effect of melatonin.Toxic.43: 93-100.

Mozaffarian, D.; Hao, T.; Rimm, E.B.; Willett, W.C.and H u, F.B. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. N Engl. J. Med.;364:2392–404.

-Naziroglu, M.; Çay, M.; Ustündag, B.; Aksakal , M. and Yekeler , H. (1999). Protective effects of vitamin E on carbon tetrachloride-induced liver damage in rats. *Cell Biochemistry and Function*; 17 (4): 253–259.

-Ozilgen M; Ozdemir M (2001). A review on grain and nut deterioration and design of the dryers for safe storage with special reference to Turkish hazelnuts. Critical Reviews in Food Science & Nutrition, 41(2), 95–132

-Park, D. L.; Nesheim, S.; Trucksess, M. W.; Stack, M. E. and Newell, R. F. (1990). Liquid chromatographic method for determination of aflatoxins B1, B2, G1 and G2 incorn and peanut products: collaborative study. Journal of the Associationof Official Analytical Chemists, 73(2), 260–266.

-Penny M Kris-Etherton and Peter L. Bordi, (2015). Effects of Daily Almond Consumption on Cardiometabolic Risk and Abdominal Adiposity in Healthy Adults With Elevated LDL-Cholesterol: A Randomized Controlled Trial

-Pomeranz Y, (1992) Biochemical, functional and nutritional changes during storage. In: Sauer DB (ed) Storage of cereal grains and their products. American Association of Cereal Chemists Inc., St. Paul, Minnesota, pp 55-141

-Rahmianna AA, Taufiq A, Yusnawan E (2007) Effect of harvest timing and postharvest storage conditions on aflatoxin contamination in groundnuts harvested from the Wonogiri regency in Indonesia. SAT ejournal 5:1-3

-Rastogi, R., A.K and K. A. Rastogi, (2001). Longtermeffectof aflatoxinB1on peroxidation in rat liver and kidney:Effect of picroliv and silymarin. Phyt. Res.15:307-310.

-Reed KA, Sims CA, Gorbert DW, O'Keefe SF, (2002). Storage water activity affects flavor fade in high and normal oleic peanuts. Food Res Int 35:769-774.

-Reeves, P. G.; Nielsen, F. H. and Fahmy, G. C. (1993): Reported of the American Institute of Nutrition Adhoc Wriling Committee on the reformulation of the AIN-76 A Rodent diet. *J. Nutr., 123:1939-1951*

-Reitman, S.; and Frankel, S. (1957): Enzymatic determination of liver function. Am. J. Clin. Path. 28-56.

-Sakata, T.; Yoshimatsu, H.; Masaki, T. & Tsuda, K. (2003). Antiobesity actions of mastication driven by histamine neurons in rats. *Exp. Biol. Med. (Maywood)*; 228: 1106–1110.

-Schatzki T F; Ong M S (2001). Dependence of aflatoxin in almonds on the type and amount of insect damage. Journal of Agricultural and Food Chemistry, 44, 4513–4519.

-Shadbad, M.R.S., M. Ansarin., A .Tahavori, F. Ghaderi, and M. Nemati, (2012). Determination of Aflatoxins in nuts of Tabriz confectionaries by ELISA and HPLC methods. Ad Pharm Bull. 2 : 123-126.

-Shank, R.C.,Wogan G.N., Gibson J. B. and Nondasuta A. (1972). Dietary aflatoxin and human liver cancer. II Aflatoxin in market foods and foodstuffs of Thailand and Hong Kong. Food and Cosmetic Toxicology 10(1): 61-69.

-Singh, P.K and A.N. Shukla (2008). Survey of Mycoflora Counts, Aflatoxin Production and induced biochemical changes in walnut kernels. J Stored Prod. Res. 44:169-172.

-Slavin, J.L. (2005). Dietary fiber and body weight. *Nutrition;* 21: 411–418.

-Soni, M.; Mohanthy, P.K. and Jaliwala, Y.A. (2011). Hepato protective activity of the fruits of Prunus. *International Journal of Pharma and Biosciences.* ; 2 (2): 439-452.

-Wang, J., and X.M. Liu (2006). Surveillance on contamination of total aflatoxins in corn, pea nut, rice, walnut and pine nut in several areas in China. Chinese J Prev Med. 40:33-37.

-Wesolek, N., and A.C. Roudot (2014).Monte Carlo Modeling Aflatoxin B1Distribution Pistachio Samples: Prerequisite For Sampling Pant Validation. J. Food .Tech. Res.1:1-20

-**Williams JH (2008).** Institutional stakeholders in mycotoxin issues - past, present and future. In: Leslie JF, Bandyopadhyay R, Visconti A (eds) Mycotoxins: Detection methods, management, public health and agricultural trade. CAB International, Oxfordshire, pp 349–358.

-**Yang, J.; Liu, R.H. and Halim, L. (2009)**. Antioxidant and antiproliferative activities of common edible nut seeds. LTW—*Food Science and Technology* 42, 1–8.

-**Zhao Penga,b, Liangkai Chena,b, Yalun Zhua,b, Yue Huanga,b, Xiaoli Hua,b, Qinghua Wuc, Andreas K. Nüsslerd, Liegang Liua,b, Wei Yanga,b 2018.** Current major degradation methods for aflatoxins: A review Trends in Food Science & Technology 80 (2018) 155–166∗،

-**Zubair ,A., Z. Ud-Din, Saleemullah, S. A. Khan, H.U.Shah B.A.Khan, and E. Ali (2011).** Fatty Acid Profile and Aflatoxin Contamination of Walnut (Juglansregia L). Arp. J. Agri Biol Sci. 6:1-8.