RESEARCH ARTICLE

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EFFECT OF DETOXIFICATION OF AFLATOXINS BY OZONE OR HOT AIR ON ALBINO RATS

ABSTRACT:

Aflatoxins (Afs) are always produced during grains and wheat storage, creating severe health problems when ingested. This study aims to test the use of the ozone and hot air for the treatments of the toxicity of aflatoxins contaminated diet to albino rats. Aflatoxins production by *Aspergillus parasiticus* (2×10^4 spores/kg) were decreased in diets treated with either ozone or hot air compared with un-treated diet. Animals fed on aflatoxins contaminated diet alone showed a significantly decreased feed intake and body weight compared with control. Animals fed on Afs-contaminated diet and treated with O_3 or hot air showed significantly higher feed intake and body weight than those of Afs fed group. Significant changes in serum biochemical parameters were recorded for rats fed on Afs-contaminated diet compared with those of the control group. Similarly, rats of Afs-contaminated group exhibited severe histological anomalies of the tested organs (liver and kidney). The recorded histological alterations in the liver were liver cells degeneration, necrotic nuclei and leucocytic infiltration while those in the kidney were congestion in the glomerulus and vacuolar degeneration in the epithelium of renal tubules. The recorded data showed improvement of biochemical parameters as well as histological profiles of liver and kidney of rats fed on Afs-contaminated diet and treated with either ozone or hot air. Thus, it could be concluded that ozone and hot air induced protective effect against physiological and histological changes induced by aflatoxins. It was found that ozone was more efficient than hot air treatment.

KEY WORDS:

Aspergillus parasiticus, Aflatoxins, Hot air, Ozone, Rats

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INTRODUCTION:

Aflatoxins are found as contaminants in human and animal food as a result of fungal contamination both pre and post-harvest, with the rate and degree of contamination being dependent on temperature, humidity, soil and storage conditions (Abdul-Ahad and Al-khateeb, 2006). Superficial contamination of seed for human or animal feeding during storage and marketing derives from infections produced in field and from saprophytic fungi as *Aspergillus* spp. *Fusarium* spp. and *Penicillium* spp. that produce dangerous metabolites for human or animal health (mycotoxins producer) (Ciccarese *et al.*, 2007). Aflatoxin B1, B2, G1, G2, M1, ocratoxin A, zearalenone, and fumonisin cause diseases or illness when eaten by

humans and animals (Sarwar and Khalil, 2005).

Activity of mould fungi *Aspergillus flavus* can be inhibited by grain exposure to 5 ppm ozone concentration (Mason *et al.*, 1997). Several studies undertaken previously had established the effectiveness of ozonation as a decontamination process. It has been found to be effective in reducing aflatoxin levels by as much as 95% (Prudente and King, 2002). Ozonation was also used to quantify aflatoxin destruction compared with untreated samples. The ozonation process resulted in 56 - 77% reduction of AFB1 and 61 - 80% reduction in AFB2. Proctor *et al.* (2004) and Akbas and Ozdemir (2006) reported that 51% degradation of both AFB_2 and AFG_2 in peanut kernels. For peanut flour, 20% and 30% degradation was observed for AFB_2 and AFG_2, respectively. This suggests that ozonation at room temperature for 10 – 15 minutes could yield degradation levels similar to those achieved at higher temperatures while being more economical. Ozonation process reduced total aflatoxin and AFB_1 by 24% and 23%, respectively, for pistachio kernels and only 5% for ground pistachios. The process reduced the aflatoxin B_1 content in flaked red pepper by as much as 80% after ozonation for 60 min. Further, the level of aflatoxin B_1 in crush red pepper with moisture content of 12.7% and initial aflatoxin B_1 level of 32 ppb was reduced by as much as 93% after exposure to gaseous ozone for 60 minutes (Inan *et al.* 2007). Ozonation (12-13 wt %) totally degraded aflatoxin B_1 in a model system. Conversion of aflatoxin into polar compounds was observed during ozonolysis of 100 Ig aflatoxin B_1 in an aqueous environment and in solid form. Ozonation (9 - 10 wt %) resulted in 74% and 44% reduction of AFB_1 and AFB_2 levels, respectively (Prudente, 2008). Ozone is a strong oxidant effective in controlling bacteria, molds, protozoa, and viruses. Ozone decays more than 99.99% of fungus, moss, and bacteria within 10 seconds. Ozone can be used for disinfection, cleansing, and deodorization without side effects**.** Ozone might have different applications, such as cleaning surfaces or equipment and disinfecting water for recycling (Jeong, 2010).

Heat treatments have also been very effective in controlling fungi that are the main causes of postharvest decay development (Vicente *et al*., 2002). Heat treatments against pathogens may be applied to freshly harvested produce in several ways: by vapour heat, hot dry air, hot water dips (Fallik, 2004). Hot air oven drying of the diet resulted in an average reduction of 57.6% in aflatoxin content, whereas sun drying reduced the aflatoxin content by 83.7%. It can be concluded that drying of feed either in hot air oven (80°C / 6 h) or in sunlight (14 h) is effective in reducing the aflatoxin level (Mani *et al.,* 1997).

Treatment with AF resulted in a significant increase in ALT, AST, cholesterol, triglycerides, uric acid, TNFa, LPO, NO, and CEA, whereas it decrease significantly GPX and SOD. The histopatholgical examination of the liver, kidney and testis showed sever histological changes typical to those reported for aflatoxicosis (Abdel-Wahhab *et al.,* 2007). Aflatoxins are hepatotoxic, hepatocarcinogenic, mutagenic and teratogenic. Acute aflatoxin poisoning caused hepatocellular necrosis and derangement of hepatic functions. Sub acute or chronic aflatoxicosis caused fatty changes in the liver, enlargement of the gall bladder and periportal fibrosis with proliferative changes in bile duct epithelium (Jha *et al*., 2012). AFG_1 caused significant accumulation of only neutral fat in the liver, a slight rise in serum triglycerides and intensified hepatorenal inflammation, necrosis and bile duct proliferation. AFB_1 caused the accumulation of both neutral fat and fatty acids in the liver, and was cytotoxic to the liver and kidney.

Rats and mice differ markedly in sensitivity to AFB_1 hepatocarcinogenicity, the former being sensitive and the latter resistant. The range of levels reported for AFB_1 was from 0.0 to 30 µg/kg and for total from 0 to 50 µg/kg**.** Aflatoxins induced cell shrinkage, chromatin condensation, membrane blebbing, and cell apoptosis (Abdul-Ahad and Al-khateeb, 2006). Infection of rats with *A. flavus* or intoxication with AFB_1 significantly induced renal damage as indicated by marked increased levels of serum urea, uric acid and creatinine, as well as histopathological pictures compared with normal healthy rats. Oral co-administration of aqueous extract of pumpkin fruits (1.0 mg / kg BW) to either rat groups infected with *A. flavus* or intoxicated with AFB_1 for 20 consecutive days effectively normalized the serum kidney function biomarkers and confirmed by histomorphologic pictures which showed normal histological structure (Saddiq, 2012).

The aim of this work was to examine the effect of detoxification of aflatoxins i.e. B_1, B_2, G_1, and G_2 by gaseous ozone exposure, in addition heat treatment compared with untreated (control), bio-assay testing of these toxins on small albino rats.

MATERIAL AND METHODS:

Chemicals and kits:

Serum biochemical analyses: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-Glutamyl Transferase (γ-GT) alkaline phosphatase (ALP), urea, uric acid, creatinine kits were purchased from Biomerieux, Laboratory of Reagents and Products (France). Aflatoxin B_1,

B_2, G_1, G_2, fumonisin (FB_1) and ochratoxin standards were purchased from sigma, Chemical Co (St. Louis, MO, U.S.A.).

Contaminated wheat grains with aflatoxins (Afs):

Twenty kilograms of pesticide-free wheat were inoculated with spores of *Aspergillus parasiticus*, strain (No.54) (2×10^4 spores/kg) which was isolated from wheat grains on Potato dextrose agar (PDA medium) and identified in Plant Pathology Dept., National Research Centre (NRC), El-Dokki, Egypt (Embaby *et al.,* 2012). The grains were incubated at 25 ± 2ºC for three weeks, with a daily rotation; then, they were sterilized using an autoclave (1.5 kg/cm^3 for 1 h). The liberated aflatoxins AFs i.e. B_1 (AFB_1), B_2 (AFB_2), and G_1 (AFG_1) was quantified with HPLC equipment under the following conditions: fluorometric detector (Waters 420) equipped with excitation and emission monochromators at a wavelength of 360 and 440 nm, respectively, a mixture of acetonitrile-water (1:1) as mobile phase, and a C-8 column (particle size = 5 m, id = 10 mm, and Tr = 8 min) (Madrigal- Santillán *et al*., 2006).

Experimental design:

Available Sprague-Dawley female rats (Three-month old nearly 110 ± 14.14 g) were obtained from Animal house, National Research. Centre (NRC), Cairo, Egypt. Animals were divided randomly into four groups, 5 animals each. The animals were housed in clean polypropylene cages (five individuals per cage) and maintained in an air-conditioned animal house at 23 ± 2°C. with 12 h light–dark cycle, controlled humidity and circulation of air 50 ± 10% humidity and fed commercial diet (ISO 9001 certified laboratory feed Labofeed H). Food consumption was approximately 15 g/female/day according to Jodynis-Liebert *et al.* (2006). They were allowed to freely consume tap water and were fed according to the indicated experimental diets. The animals were adapted to the environmental conditions for 7 days prior to the start of the experiment. Measurements of body weight were monitored every day throughout the 3 week exposure. The diets of the studied animals were prepared with wheat (90%) and the recommended mixture of vitamins, minerals, and proteins; then, the selected concentrations of AFs and/or wheat treated with ozone and hot air were added to the diet. The following groups of rats were established:

(a)- Animals fed on uncontaminated wheat (free aflatoxins AFs),

(b)- Animals fed on wheat contaminated with aflatoxins AFs produced by *Aspergillus parasiticus* (123 µg/kg BW).

(c)- Animals fed on AFs contaminated wheat treated by ozone 50 ppm for 15 min.

(d)- Animals fed on AFs contaminated wheat treated by thermal hot air oven at 80^{o}C for 6h.

The experimental determinations were made in rat fed with diets that included AFs three weeks with the same basic according. The weight of each rat was determined at the end of every day of the experiment (Madrigal-Santillán *et al*., 2006).

Blood samples:

All animals were not fed on the day of the sacrifice. After the experimental period (21 days), the blood samples were collected from retro-orbital venous plexus for biochemical analysis in 15 ml-polypropylene tubes containing heparin as anticoagulant and centrifuged at 3000 rpm for 10 min to separate the plasma and used for biochemical serum analysis of kidney and liver function. After blood collection, rats of each group were sacrificed under ether anesthesia and the kidney and liver samples were collected for histopathological examination, all rats were killed using ether and sacrificed (with painless) by decapitation (guillotine). Dissection was then performed and the kidneys as well as the liver were removed, weighed and immediately frozen in liquid nitrogen (freeze clamping). All samples were stored at - 80°C until analysis (Aoudia *et al.,* 2008).

Biochemical serum analysis:

The serum was diluted and used for various biochemical analysis such as serum alanine aminotransaminase (ALT) ,aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) were determined according to Reitman and Frankel (1957), Uric acid and Urea level was determined using the enzymatic colorimetric method of Barham and Trinder (1972), Creatinine was determined in serum using methods of Bartels *et al*. (1972) and γ-Glutamyl Transferase (γ-GT) was determined using methods described by Henry *et al*. (1974). At the end of treatment period, and after blood samples were collected, all animals were killed and the liver and kidney tissue of each animal was dissected, weighted and samples of each organ were homogenized in phosphate buffer to control any changes of these organs (pH 7.4) to give 20% w/v homogenate.

Histopathological observations:

Kidneys and livers were dissected from rats and fixed by following standard methods of dehydration and clearing. A small piece of kidneys and livers were fixed by 10% neutral buffered formalin and then embedded into paraffin, sectioned for 5 to 6 µm thick of the control samples treated and infected (Lin *et al*., 1998). Haematoxylin and eosin stain gives clear cytoplasm differentiation and nuclear and gives good idea about histological structure of the samples of the study and reveals some histopathological changes

(Saddiq and Kalifa, 2011). The sections were stained with hematoxylin-eosin and mounted on the glass microscope slides using standard histopathological techniques then, examined by light microscopy (Omar, 2012; Saddiq, 2012).

Statistical analysis:

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller–Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P \leq 0.05$.

RESULTS AND DISCUSSION:

Detoxification of aflatoxins contaminated wheat grains by using ozone and hot air treatment:

Aflatoxins i.e. B_1, B_2, G_1, and G_2 were detoxified by using two methods i.e. ozone and hot air treatments *in vitro.* Data were recorded in table 1 and figures 1 & 2. Data in this table show that, both these treatments were found to decrease aflatoxins content comparing with un-treated control. Also data in the same table presented that, ozone treatment gave more reduction than hot air treatment which gives average reduction of total aflatoxins 65.0 and 30.9% with ozone and hot air respectively. Ozone treatment was found to reduce aflatoxin B_1 from 33.0 to zero µg/kg and gave hundred percent in reducing aflatoxin B_1. While, hot air treatment was found to decrease aflatoxin B_1 from 33.0 to 30.0 µg/kg equal 9.1 reduction percent of aflatoxin B_1. Aflatoxin B_2 was decreased from 32.0 µg/kg with un-treated control sample to 17.0 and 22.0 µg/kg with 46.9 and 31.3% reduction percent when treated with ozone and hot air, respectively. On the other hand data also show that, aflatoxin G_1 was decreased from 58.0 µg/kg with untreated control sample to 26.0 and 43.1 µg/kg with 55.2 and 43.1% reduction percent when treated with ozone and hot air, respectively. Aflatoxin G_2 not detected.

Table 1. Effect of different treatments on aflatoxin reduction rate (%)

Sample state	B_1 (µg/kg)	Reduction rate (%)	B_2 (µg/kg)	Reduction rate (%)	G_1 (µg/kg)	Reduction rate (%)	G_2 (µg/kg)	Reduction rate (%)	Total (µg/kg)	Reduction rate (%)
W/AFs	33.0	-	32.0	-	58.0	-	ND	-	123,0	-
W/AFs/O	00.0	100	17.0	46.9	26.0	55.2	ND	-	43.0	65.0
W/AFs/HA	30.0	9.1	22.0	31.3	33.0	43.1	ND	-	85.0	30.9

W/AFs =Wheat contaminated with Aflatoxins (AFs)

W/AFs/O = Wheat contaminated with Aflatoxins (AFs), and treated by ozone

W/AFs/HA= Wheat contaminated with Aflatoxins (AFs), and treated by hot air

ND=AFs not detected

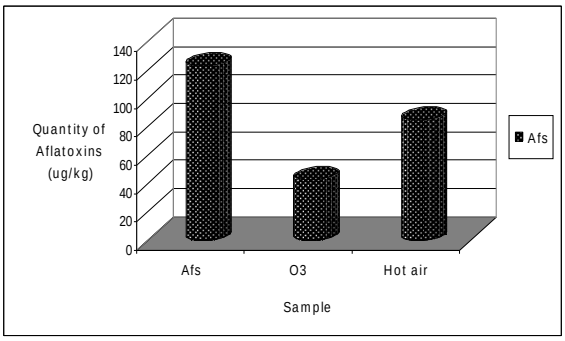

Fig. 1. Effect of different treatments on aflatoxins conc.

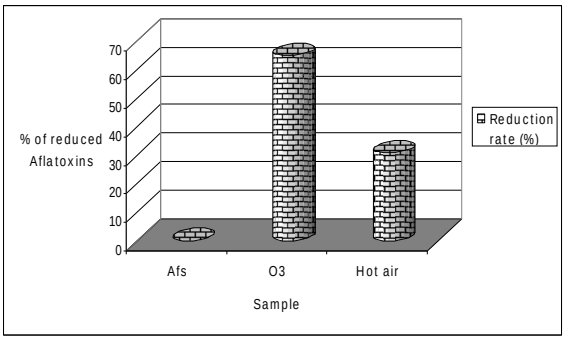

Fig. 2. Effect of different treatments on aflatoxin reduction rate %.

Jeong (2010) reported that ozone is a strong oxidant effective in controlling bacteria, molds, protozoa, and viruses. Ozone decays more than 99.99% of fungus, moss, and bacteria within 10 seconds. Ozone can be used for disinfection, cleansing, and deodorization without side effects. Ozone might have different applications, such as cleaning surfaces or equipment and disinfecting water for recycling. Also, Prudente and King (2002) reported that, Ozone treatment has been found to be effective in reducing aflatoxin levels by as much as 95%. Proctor *et al.* (2004) stated that, the ozonation process resulted in 56-77% reduction of AFB_1 and 61-80% reduction in AFB_2. On the other hand, they observed that 51% degradation of both AFB_2 and AFG_2 in peanut kernels. For peanut flour, 20% and 30% degradation was observed for AFB_2 and AFG_2, respectively. Regardless of treatment combinations, aflatoxins B_1 and G_1 exhibited the highest degradation levels. Moreover, higher levels of toxin degradation were achieved in peanut kernels than in flour. Akbas and Ozdemir (2006) found that the ozonation process reduced total aflatoxin and AFB_1 by 24% and 23 %, respectively, for pistachio kernels and only 5% for ground

pistachios. The process reduced the aflatoxin B_1 content in flaked red pepper by as much as 80% after ozonation for 60 min. Further, the level of aflatoxin B_1 in crush red pepper with moisture content of 12.7% and initial aflatoxin B_1 level of 32 ppb was reduced by as much as 93% after exposure to gaseous ozone for 60 minutes. In addition, no significant change in color between ozonated and nonozonated samples was observed using the Hunter color parameters "L, a, and b" (Inan *et al.* 2007). Ozonation (9 - 10 wt %) resulted in 74% and 44% reduction of AFB_1 and AFB_2 levels, respectively (Prudente, 2008).

Heat treatments have also been very effective in controlling fungi that are the main causes of postharvest decay development (Vicente *et al*., 2002). Heat treatment against pathogens was applied in several ways: by vapour heat, hot dry air, hot water dips (or a short, hot water rinsing and brushing (Fallik, 2004). Hot air oven drying of the diet resulted in an average reduction of 57.6% in aflatoxin content, whereas sun drying reduced the aflatoxin content by 83.7% (Gowda *et al.,* 2007).

Effect of ozone and hot air treatments on feed intake and body weight gain of rats fed on aflatoxins-contaminated diet:

Effect of both aflatoxins (AFs) alone and treated with ozone or hot air and its effect on daily feed intake and body weight(g) of experimental rats in the different treatment groups were tabulated in table 2 and figure 3 & 4. Results illustrated that, no mortality with any treatment at the end of experiment (after 21 days). Also, data indicated that all of animals fed on AFs-contaminated wheat and contaminated wheat treated with ozone (50 ppm) or hot air (80ºC) significantly ($P \leq 0.05$) decreased the feed intake and body weight comparing with un-treated control (animals feed on wheat free of AFs). Also a significant difference was found between feed intake of animals fed on contaminated wheat with Afs and contaminated wheat treated with ozone or hot air. No significant difference was found between body weights of animals fed on AFs-contaminated wheat and contaminated wheat treated with ozone or hot air. The data showed that control group has a higher feed intake and body weight followed by ozone and hot air groups respectively, while aflatoxins (AFs) group has the lowest feed intake and body weight. The average of feed intake of animals for control group was 113.5 (g) which decreased to 85.6 and 67.6 (g) with animals fed on contaminated wheat treated with ozone and hot air respectively. AFs -contaminated diet group had the lowest feed intake 50.1 (g).While the average of body weight of animals for control group was 640 g which decreasing to 370.7 g with animal's rat fed with AFs-contaminated wheat group while the groups fed on AFs - contaminated wheat treated with ozone or hot air improved in total body weight to be 477.8 and 427.1 gm respectively. Similar results were obtained by Abdel-Wahhab *et al*. (2010), they found that, ingestion of AFB_1 mycotoxin resulted in a significant decrease in feed intake. Also, Farag *et al.* (1996) and Mani *et al.* (1997) reported that, average feed intake was lower in sheep fed on the diet without drying in hot air oven (80ºC/ 6 h) or in sunlight (14 h). Sherif *et al*. (2012) stated that, administration of AFB1 induced significant decrease in body weight of rats.

Table 2. Effect of ozone and hot air treatments on body weight gain and feed intake of rats fed on aflatoxins-contaminated diet for three weeks (means ± SE)

parameters \ Groups	Control (g)	W/AFs (g)	W/AFs/O (g)	W/AFs/HA (g)
Feed Intake	113.5 ± 1.21 [a]	50.1 ± 1.62 [d]	85.6 ± 0.58 [b]	67.6 ± 0.42 [c]
Body weight	640 ± 53.45 [a]	370.7 ± 16.98 [b]	477.8 ± 21.20 [b]	427.1 ± 8.0 [b]

* Means superscript with different letter were significantly different ($P \leq 0.05$).
Control = Wheat free of Aflatoxins (AFs).
W/AFs = Wheat contaminated with Aflatoxins (AFs)
W/AFs/O = Wheat contaminated with Aflatoxins (AFs), then treated by ozone
W/AFs/HA= Wheat contaminated with Aflatoxins (AFs), then treated by hot air

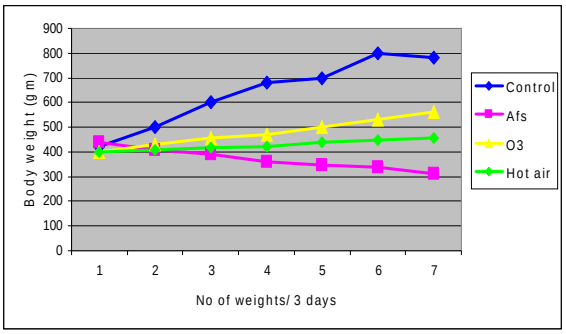

Fig. 3. Effect of AFs and its treatments on total body weight (g).

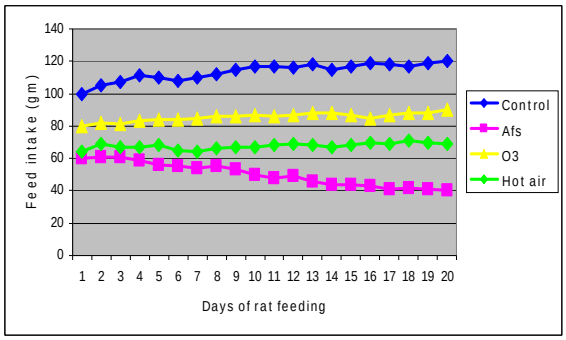

Fig. 4. Effect of AFs and its treatments on daily feed intake of rats (g).

Biochemical results

The effects of aflatoxins alone and treated with ozone or hot air on serum biochemical parameters were recorded in table 3 and figures 5 & 6. Data indicated that control group was in normal limit, but when rats fed on wheat contaminated with AFs induced significantly ($P \leq 0.05$) increasing all biochemical parameters i.e (urea, creatinin, uric acid, ALP, ALT, AST, and GGT). It was worthy to mention that rats fed on contaminated treated wheat with either O_3 or hot air restore all biochemical parameters towards the control group and treatment with O_3 better than treatment with hot air in the respect of biochemical parameters. Similar results were obtained by Saddiq (2012), she reported that infection of rats with *A. flavus* or intoxication with AFB_1 significantly induced renal damage as indicated by marked increased levels of serum urea, uric acid and creatinine. Sherif *et al*. (2012) found that, the activities of serum marker enzymes ALT, AST, GGT, ALP, creatinin and uric acid significantly increased with orally administration of AFB_1. Abdel-Wahhab *et al*. (2007) reported that, the treatment with AFs resulted in a significant increase in ALT, AST, cholesterol, triglycerides, uric acid, TNFa, lipid peroxidation, uric acid, and creatinine.

Table 3. Effect of Afs and both ozone and hot air treatments on serum biochemical parameters of rats (Mean ± Sd)

Biochemical parameter / Tested group	Urea (mg/dl)	Creat (mg/dl)	U.A (mg/dl)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)
Control	58.66 ±2.02 [a]	0.88 ±0.07 [a]	1.53 ±0.14 [a]	56.33 ±4.05 [a]	25.0 ±1.0 [a]	28.0 ±0.57 [a]	50.0 ±2.64 [a]
W/AFs	68.33 ±0.66 [b]	1.40 ±0.05 [b]	2.43 ±0.14 [b]	61.66 ±5.69 [a]	31.66 ±1.33 [b]	38.0 ±1.0 [b]	55.66 ±1.20 [a]
W/AFs/O	59.66 ±0.33 [a]	0.96 ±0.03 [a]	1.26 ±0.14 [a]	50.0 ±0.57 [a]	28.33 ±1.66 [a b]	34.0 ±0.57 [c]	51.33 ±0.88 [a]
W/AFs/HA	61.66 ±0.88 [a]	1.04 ±0.08 [a]	1.23 ±0.14 [a]	52.66 ±0.33 [a]	29.00 ±0.0 [a b]	35.33 ±0.33 [b c]	52.66 ±0.33 [a]

* Within each column, means superscript with different letter were significantly different ($P \leq 0.05$).

Control = Wheat free of Aflatoxins (AFs).

W/AFs = Wheat contaminated with Aflatoxins (AFs)

W/AFs/O = Wheat contaminated with Aflatoxins (AFs), then treated by ozone

W/AFs/HA= Wheat contaminated with Aflatoxins (AFs), then treated by hot air.

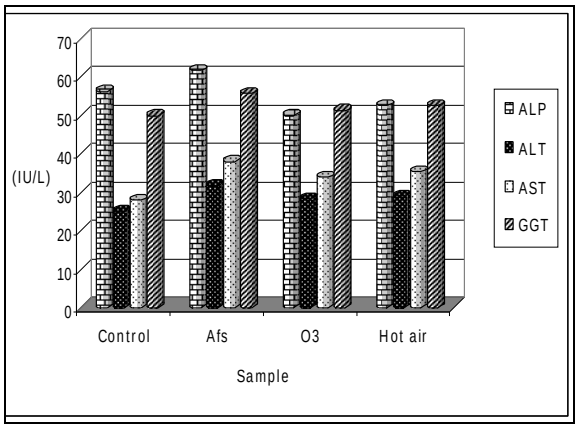

Fig. 5. Effect of Afs and treatments with ozone & hot air on serum kidney functions of rats

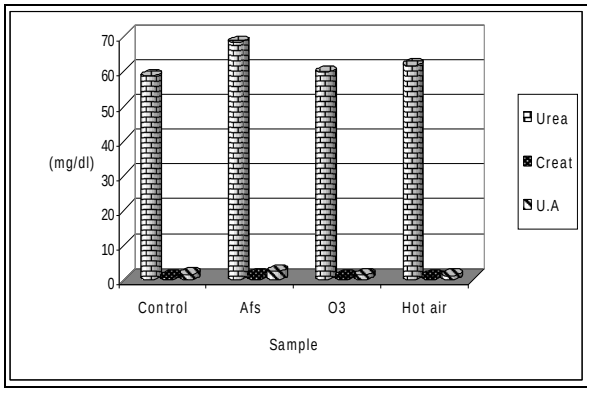

Fig. 6. Effect of Afs and treatments with ozone & hot air on serum liver functions of rats

Histopathological changes:

Liver of rat control group showed normal histological structure of hepatic lobule (Fig. 7), while liver of AFs group appeared with focal hepatic necrosis associated with leucocytic cells infiltration (Fig. 8). Slight liver dilatation (Fig. 9) and congestion of hepatic sinusoids (Fig. 10) were observed in rats fed on Afs contaminated diet and treated with ozone and hot air, respectively. This indicates that treatment with O_3 or hot air improves the histological picture of rats.

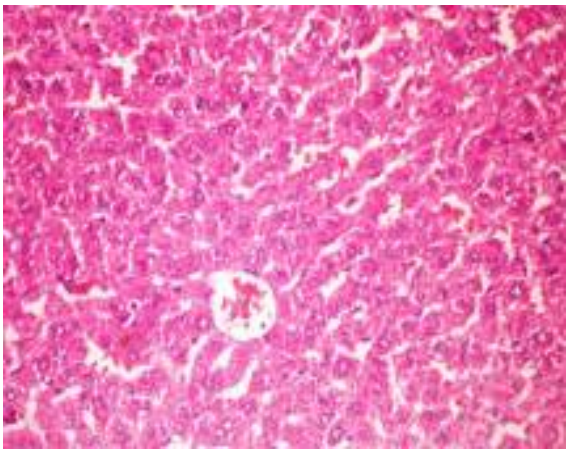

Fig. 7. Liver section of rat from control group showing the normal histological structure of hepatic lobule. X 400.

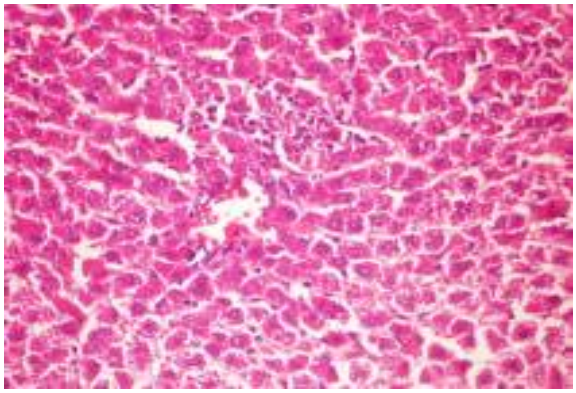

Fig. 8. Liver section of rat from group AFs showing focal hepatic necrosis (arrow) associated with leucocytic cells infiltration. X 400.

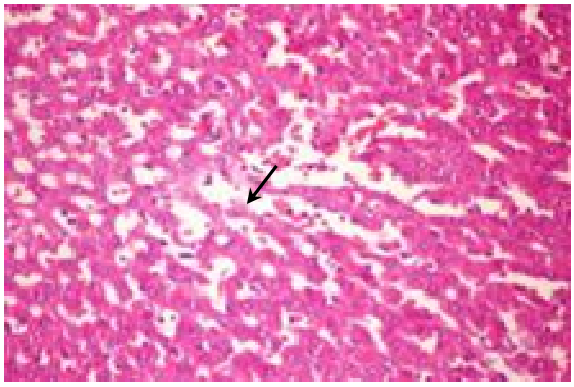

Fig. 9. Liver section of rat from O3 group (50 ppm) showing slight dilatation and congestion of hepatic sinusoids. X 400.

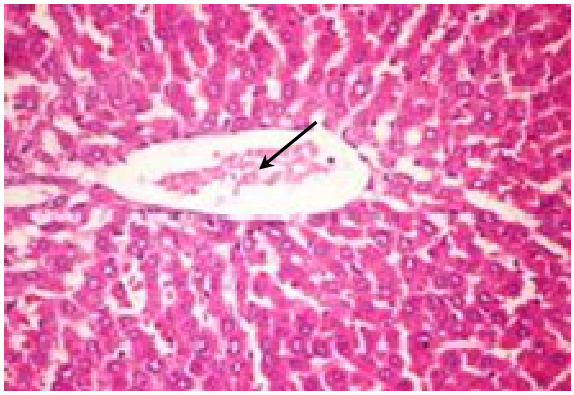

Fig. 10. Liver section of rat from hot air group showing slight dilatation of hepatic sinusoids. X 400.

The histological examination of kidney of control group gave normal histological structure of renal parenchyma (Fig. 11), while kidney of AFs group showed congestion of glomerular tufts and vacuolar degeneration of epithelial lining renal tubules (Fig. 12). Animals given Afs treated with ozone or hot air showed slight change. The kidney tubules showed slight vacuolar degeneration and the glomerului showed slight congestion (Figs 13 & 14). Similar results were obtained by Ward and Dally (2002) and Ahsan *et al*. (2009); they found that histopathological analysis of the liver and kidney of rats fed on AFs-contaminated diets revealed different lesions in tissues. Abdel-Wahhab *et al*. (2007) reported that, the histopatholgical examination of the liver, kidney and testis showed several histological changes typical to those reported for aflatoxicosis**.** Hoerr and D'Andrea (1983) stated that epidemiological survey indicated that occurrence of hepatic and kidney disorders due to aflatoxins increased as life style changes causing various pathological effects on organs and tissues. Also, Saddiq (2012) reported that infection of rats with *A. flavus* or intoxication with AFB_1 significantly induced renal damage as indicated by histopathological pictures compared with normal healthy rats.

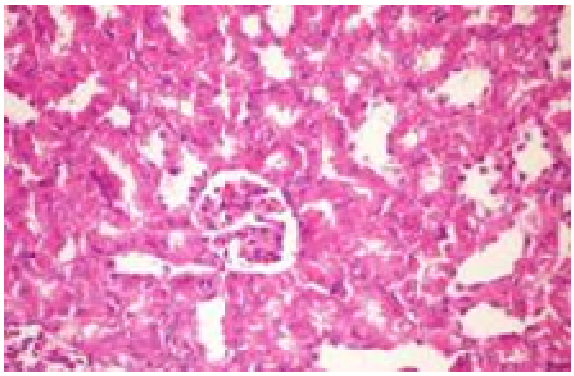

Fig. 11. Kidney section of rat from control group showing the normal histological structure of renal parenchyma. X 400.

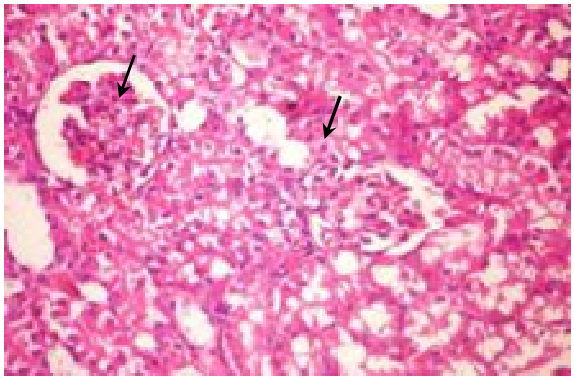

Fig. 12. Kidney section of rat from AFs group showing congestion of glomerular tufts and vacuolar degeneration of epithelial lining renal tubules. X 400.

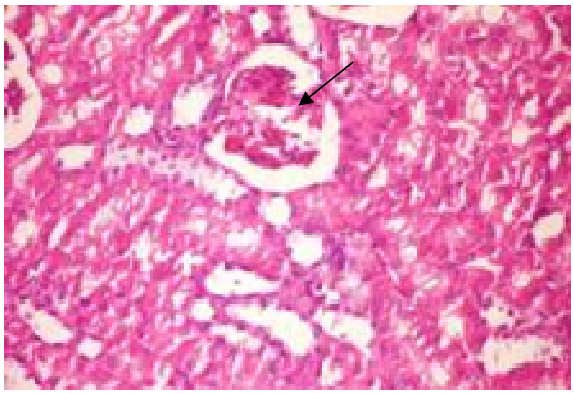

Fig. 13. Kidney section of rat from O3 group (50 ppm) showing slight congestion of glomerular tuft. X 400.

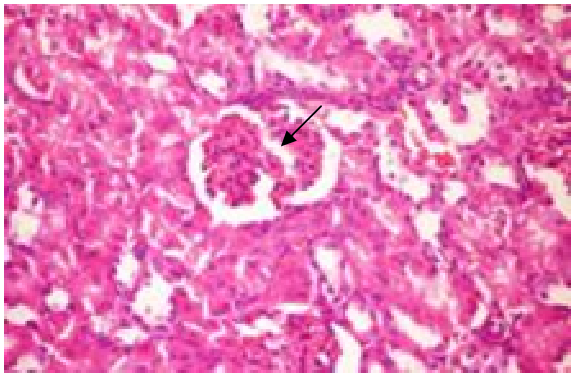

Fig. 14. Kidney section of rat from hot air group showing congestion of glomerular tuft. X 400.

CONCLUSION:

Both hot air and ozone treatments were found to decreased aflatoxins risk hazard of animals feeds and enhance the histological effects.

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تأثير إزالة سمية الأفلاتوكسين باستخدام المعالجة بالأوزون والهواء الساخن على الجرذان البيضاء

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السموم الفطرية والتى تنقسم إلى B1, B2, G1, G2 حيث تفرز نتيجة للتعفن بفطر الأسبرجلس براسيتيكس. وفى هذا تم معالجة أثارها السمية وذلك بمعالجة القمح الملوث بها بكل من الأوزون والهواء الساخن. وتوصلت النتائج إلى أن هذة المعالجة تؤدى إلى تقليل كمية السموم الفطريه عن نظيرها فى المجموعة غير المعالجة. كما تم إجراء تغذية الجرذ الأبيض على كل من الأغذية الملوثة بالسموم الفطريه والمعالجة بالأوزون أو الهواء الساخن وكذلك على تلك الغير معالجة وذلك لأختبار أثارها السامه على بعض الأنشطة الحيويه والتراكيب الهستولوجية. وأوضحت النتائج حدوث نقصان شديد فى الوزن الكلى للجرذان التى تغذت على غذاء ملوث بالسموم الفطرية والغير معالج. كما اشارت النتائج إلى تغيرات بيوكيميائية شديدة بالأضافة إلى تهتك فى أنسجة الكبد والكليه لجرذان تلك المجموعة. وعلى الجانب الأخر أوضحت الدراسة تحسن فى المعايير البيوكيميائية والهستوباثولوجية لجرذان المجموعة المغذاة على أغذية ملوثة بالسموم الفطرية ومعالجة بالأوزون والهواء الساخن. لذلك خلصت هذة الدراسه على فاعلية المعالجة بالأوزون والهواء الساخن لإزالة سمية السموم الفطرية وفاعلية المعالجة بالأوزون كانت أفضل.