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Research Article

INFLUENCE OF DEXYNIVALENOL OR FUMONISIN B1 TOXIC EFFECT-INDUCED BY ZEARALENONE FOLLOWING SHORT-TERM REPETITIVE ORAL ADMINISTRATION TO FEMALE SWISS MICE

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ABSTRACT
The natural co-occurrence of *Fusarium* toxins zearalenone (ZEA), fumonisin B_1 (FB_1) and deoxynivalenol (DON) in the same food was well known. Previous *in vitro* studies on their interactive effect had reported additive or synergistic effect but interestingly antagonistic effect. In order to explore *in vivo* such characteristic interactive effect, Swiss female mice were orally administered with low doses of ZEA (50µg/kg bw/day), FB_1 (110µg/kg bw/day) or DON (45µg/kg bw/day) alone and their mixtures (ZEA+FB_1) and (ZEA+DON) for 7 days. As results, no death or abnormal symptoms were observed in all groups. The significant of loss of weight was observed in female mice group treated with FB_1 or DON and their associations with ZEA. Serum chemistry examinations revealed disorders in lipid metabolism, renal filtration and a rhabdomyolysis. ZEA tested at dose of 50µg/kg bw/day was very slightly toxic or not but associated with FB_1 or DON, enhanced their effect in particular in renal filtration failure, tissue necrosis and lipid metabolism troubles. These combined effect suggested an additive or unexpected synergistic effect in female mice. Moreover, ZEA+DON were more potent in tissue necrosis but in renal filtration and troubles in lipid metabolism, the binary ZEA+FB_1 had been found alarming. These toxic effects could be regarded as precocious effects of ZEA, DON and FB_1 since they had been tested at very low doses. Thus, the no-observed effect levels (NOAEL) of ZEA, DON and FB_1 were low than 50µg/kg bw/day, 45µg/kg bw/day and 110µg/kg bw/day respectively above all when they were simultaneously administrated.

Key Words: *Fusarium Toxins, Interactive Effect, Female Mice*

INTRODUCTION
Fusarium species occur widely on plants. They are found in a variety of agricultural products mainly on corn, wheat and other cereal grains for human and animal consumption. *Fusarium* toxins namely deoxynivalenol (DON), zearalenone (ZEA) and fumonisin B_1 (FB_1) have been shown to cause diverse toxic effects in both experimental animals and livestock and occasionally suspected to cause adverse effects in humans (Gelderblom and Marasas, 2012; Creppy, 2002). These mycotoxins could be found in the same commodities as secondary metabolites of different *Fusarium* species (Desjardins *et al.*, 2000; Dawlatana *et al.*, 2002). Thus, several studies have been focused on their interactive effect in human or animal cells lines (Kubena *et al.*, 1997; Boeira *et al.*, 2000; Tajima *et al.*, 2002; Creppy *et al.*, 2004; Kouadio *et al.*, 2007). In previous in vitro study focused on binary of ZEA and FB_1 or binary ZEA and DON using several endpoints such as lipid peroxidation, protein and DNA synthesis, cell viability, DNA methylation and DNA fragmentation, we have reveled FB_1 and ZEA display antagonism or additive effects in Lipid peroxidation, inhibition of protein synthesis, DNA methylation and fragmentation while on the association of ZEA and DON lead to globally to additive effects (Kouadio *et al.*, 2007). These findings should be confirmed by in vivo studies.
Concerning the individual toxic effects of these major *Fusarium* mycotoxins, several studies and epidemiological reports revealed that fumonisin B_1 (FB_1) causes equine leukoencephalomalacia, porcine

pulmonary oedema, nephrotoxicity, hepatotoxicity, and hepatocarcinogenicity in laboratory animals and of oesophageal carcinoma in humans (Gelderblom and Marasas, 2012; Voss *et al.*, 1998; Riley *et al.*, 2001; Ribar *et al.*, 2003). FB_1 had been also found to cause hepatotoxicity and nephrotoxicity in rabbits and lambs by increasing the rate of apoptosis in liver and kidney (Edrington *et al.*, 1995; Bucci *et al.*, 1998). For ZEA, it has estrogenic and anabolic activities in several species such as rodents, pigs and monkeys (Creppy, 2002), being able to cause alterations in the reproductive tract of laboratory animals (Abid-Essefi *et al.*, 2004). ZEA is associated with outbreaks of precocious pubertal changes in children in Puerto Rico, and has been suggested to have a possible involvement in human cervical cancer (Zinedine *et al.*, 2007). The main targets of ZEA were those tissues enriched in the estrogen receptors, liver, kidney and immune systems (Abbes *et al.*, 2006). In addition to the estrogen function, it also could cause tissue oxidative stress (Hou *et al.*, 2013). ZEA was found to have hepato-nephrotoxicity and could disturb the enzymatic and hematological parameters of mice within 48 h taken orally (Abbes *et al.*, 2006). ZEA could cause DNA adducts formation in female mousse tissues (Pfohl-Leszkowicz *et al.*, 1995) and was known as carcinogenic disorders (Creppy, 2002). The third Fusarium mycotoxin DON produced abundantly by *Fusarium graminearum* (Creppy, 2002) when ingested can induce a decrease in food intake or refusal to eat food, vomiting, and digestive disorders; with subsequent losses of weight gain in animals which ingest this mycotoxin (Flannery *et al.*, 2011). The gastrointestinal system is the target organ of the toxin (Young *et al.*, 1983). DON induced hematological effects, lesions in the nonglandular stomach, and caused thymic lymphoid depletion, increased incidences, and mean severity of spleenic hematopoiesis, and increased mean severity of sternal bone marrow adipocyte deposition in rats at the highest dose (Iverson *et al.*, 1995).

Although the existing studies on experimental animals such as pig and chicken revealing that tissue oxidative stress could be induced by the individual DON, ZEA or FB_1 treatment, but, most of works were based on the separated mycotoxin treatment. In contrast to the pure mycotoxin feeding experiments, consumption of naturally contaminated multiple mycotoxins *in vivo* could provide more actual information on the effects of mycotoxins on the tissue toxicity. In addition, a previous study on *in vitro* combined effect of ZEA and FB_1 or ZEA and DON had revealed additive or antagonistic cytotoxic effect (Kouadio *et al.*, 2007). Therefore, the aim of this study is to investigate on interactive effect of ZEA and FB_1 or those of ZEA and DON using an *in vivo* model. This experiment may provide data interesting to compare toxic effects resulting from binary ZEA+FB_1 or binary ZEA+DON.

MATERIALS AND METHODS

Chemicals

DON, ZEA and FB_1 were obtained from Sigma Chemical Company (St Louis, MO, USA) and were dissolved in ethanol/water (50:50). All other chemicals used were of analytical grade. For the study, the mix solvents (ethanol/water) was dried under an air stream at 50°C and reconstituted in water which became the vehicle in the experimentation.

Animals

Female Swiss mice (7–8 week old), weighing between 20 and 25 g were obtained from Society of DEPRE (France). Mice were housed in environmentally protected transparent polypropylene cages with stainless steel wire tops for a period of 1 week before induction of different treatments. The mice had free access to water. Experimental diets were placed in special containers to minimize spillage. Environmental conditions included 23–25°C, relative humidity of 45–55%, and a 12-h light: dark cycle. The protocol for this study was approved by the Committee of Bioethics of Nangui Abrogoua University, Abidjan, Côte d'Ivoire.

Animal Treatment

Thirty (30) female mice were divided into six groups (n = 5 per group): (a) Normal control group, mice received water; (b) DON dosed group, mice received 45 µg/kg bw/day of DON; (c) FB_1 dosed group, mice received 110 µg/kg bw/day of FB_1; (d) ZEA dosed group; mice received 50µg/kg bw/day of ZEA,

(e) ZEA + FB_1 dosed group, mice receive 50 (ZEA) + 110 (FB_1) µg/kg bw/day and (f) ZEA+DON dosed group; mice receive 50 (ZEA) + 45 (DON) µg/kg bw/day. Each group received the appropriate vehicle (water), ZEA, DON, FB_1, ZEA + FB_1 or ZEA + DON daily by oral administration for 7 days. Every day the weight of the mice was observed using a Sartorius balance (ED 224S Germany) with 0.0001 mg accuracy. Urine samples were collected refrigerating metabolic cages (Tecniplast, Buguggiate, Italy) and at the end of the experiment, the mice were sacrificed to collect serum and tissues.

Assay for Serum and Urine Chemistry

The serum biochemistry parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), triglyceride (TG), total cholesterol (TC), creatinine, gamma glutamyl transpeptidase (γ-GT) and creatine kinase (CK) were evaluated spectrophotometrically, using commercially available diagnostic kits supplied by BioMerieux (France). Similarly, unary total protein and creatinine were evaluated spectrophotometrically using commercially available diagnostic kits supplied by BioMerieux (France).

Renal DNA Methylation

The DNA methylation examination was performed as previously described with okadaic acid (Matias and Creppy, 1998). DNA was extracted using the Wizard genomic extraction kit (Promega, France) which is designed for isolation of DNA from white blood cells, tissue culture cells and animal tissue, plant tissue, yeast, and gram positive and gram negative bacteria. Then, DNA was quantified by ultraviolet (UV) spectrophotometry at 254 nm. Ten micrograms of purified DNA dissolved in 10 µl of water was incubated at 100°C for 2 min and then treated with 1 µl of 250 mM potassium acetate buffer (pH 5.4), 1 µl of 10 mM zinc sulfate, and 2 µl of nuclease P1 (6.25 U/µl; Sigma, France) overnight at 37°C, and then treated with 2 ml 0.5 M Tris-HCl (pH 8.3), plus 2 ml of the buffer containing alkaline phosphatase (0.31 U/µl). This mixture was incubated at 37°Celsius (37°C) for 2 h.

Analysis of DNA base composition was performed on an Instrumentation Consommable Service (ICS; Toulouse, France) chromatograph equipped with a UV Spectra Focus 3-D at room temperature using a C18-phenyl-nucleosyl column (250 × 3.4 mm). Elution was carried out with 6.5 mM H_2PO_4 (NH_4), pH 3.95, and 4% (v/v) methanol at a flow rate of 1 ml/min. Eluates were monitored at 254 nm. Standard bases (dC, dT, dG, dA) (100 µg/µl) and m5dC (10 µg/ml) were obtained from Sigma Chemical and used for quantification, after sequential injection into the high-performance liquid chromatography (HPLC) system. The surface under the curve adjusted to the known concentration of each base was used with computer aided software (Pic3, ICS, France) to quantify the bases. The results in micrograms were used to calculate the rate in percentage of m5dC as compared to m5dC + dC × 100. These rates were presented as mean of the four independents experiments ± standard error of mean (SEM), prior to statistical analysis.

Statistical Analyses of Data

Results are presented as mean ±SEM and analyzed using a nonparametric statistical test, Mann–Whitney test for significance of differences. Acceptable limit is set from $p < 0.05$* or $p< 0.01$** (Gad and Neil, 1982).

RESULTS AND DISCUSSION

The doses used in the present study were low when compared to those reported in literature. For example, in a study on induction of food refusal by DON, it has been reported that the NOAEL for DON is 0.5 and 1.0 mg/kg bw following ip. and oral exposure, respectively (Flannery *et al.,* 2011). For FB_1, the does reported in literature varied from 1 to 75 mg/kg bw (Bondy *et al.,* 1998; Motelin *et al.,* 1994) while those of ZEA were ranged from 50µg to 8mg/ kg bw (Collins *et al.,* 2006; Gajecka and Przybylska-Gornowicz, 2012). The doses of ZEA, DON and FB_1 used in the previous both studies being higher than those used in the present study, the toxic effects induced by DON and FB_1 or their mixture could be considered as precocious effects. Moreover, only female and young mice have been used in the study for their high sensibility to toxicants (Lipnick *et al.,* 1995).

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Evolution of the Weight of Mice

During the study, no remarkable changes in general appearance were observed and all mice survived until scheduled necropsy. Likewise, we observed significant decrease in body weight of mice treated with ZEA, DON, FB_1 and the mixtures ZEA+DON and ZEA+FB_1 at day 3 when compared to control as showed in Figure 1. However, only DON and the mixtures ZEA+DON and ZEA+FB_1 decreased the body weight at day 7 in mice as observed in Figure 1.

The mixture ZEA+FB_1 was more potent in the reduction of animal weight when compared to those induced by the mixture ZEA+DON. The decrease of animal weight may be explained by the reduction in food intake. But, high water depletion has been observed in animals treated by ZEA+FB_1 that could be caused the weight loss.

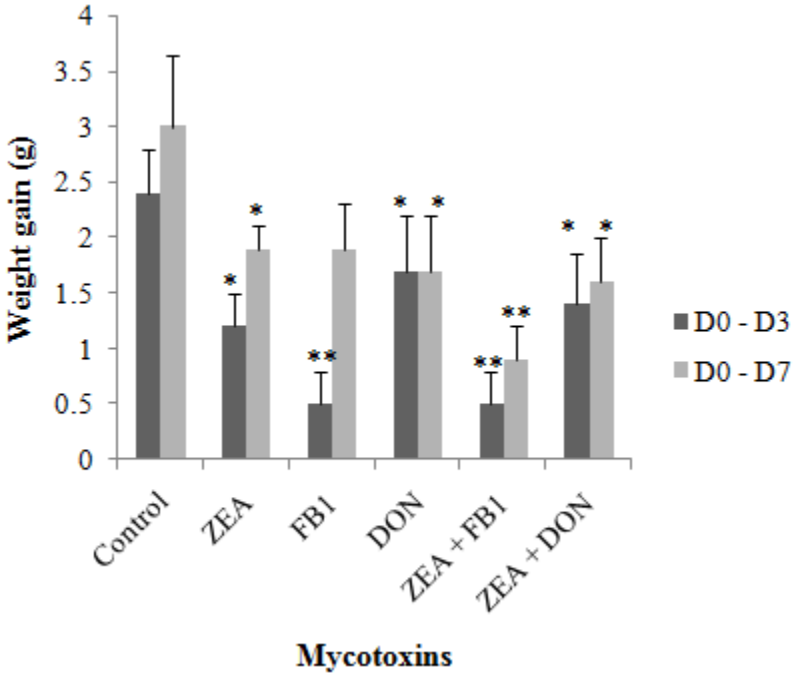

Figure 1: Evolution of body weight (g) after oral treatment female mice by zearalenone (ZEA; 50µg/kg bw/day), deoxynivalenol (DON; 45 µg/kg bw/day), fumonisin B_1 (FB_1; 110 µg/kg bw/day) and their mixtures (ZEA+FB_1; ZEA+DON); $P < 0.05$ (*)

Serum and Urine Chemistry

The results of biochemical examinations were summarized in Tables 1, 2 and 3. An elevation of triglycerides was observed in female mice treated by DON, FB_1 and their association with ZEA but the mixture ZEA+DON was more potent. In addition, the total cholesterol was significantly increased in mice treated with mycotoxins DON, FB_1 and their mixtures ZEA+FB_1 and ZEA+DON but ZEA did not influence of DON or FB_1 alone effect-induced.

ZEA and the mixture ZEA+FB_1 increased slightly serum ALT in mice but there was no difference between the mixture toxic effect and that of ZEA alone. While the individual mycotoxin ZEA or DON did not cause AST elevation but their association ZEA+DON leaded to elevation of AST and ALT in mice. For the serum CK, DON alone caused CK elevation and its association with ZEA leaded to more elevation of CK suggesting an additive effect. No modifications were observed with ZEA or FB_1 alone but their mixture slightly increased serum CK concentrations.

Moreover, no changes were observed in γ-GT serum levels in contrast to serum creatinine concentrations. Indeed, FB_1 or ZEA alone but not DON, provoked an increase of level of serum creatinine in mice and these elevations were more high with the mixtures ZEA+FB_1 or ZEA+DON. In addition, the increase of level of serum creatinine was correlated by diminishing of unary creatinine level. The similar observations were found with levels of serum total proteins and unary total proteins.

Table 1: Blood parameters for females mice after oral treatment by DON (45 μg/kg bw/day), FB_1 (110 μg/kg bw/day), ZEA (50 μg/kg bw/day) and the mixtures ZEA+FB_1 and ZEA+DON; *P* < 0.05(*)

	Cholesterol (mg/dl)	**Triglycerides (g/l)**	**ALT (IU/l)**	**AST (IU/l)**	**γ-GT (IU/l)**	**Creatine kinase (IU/l)**	**Creatinine (mg/dl)**	**Proteins (g/dl)**
Control	172 ± 4	0.89 ± 0.05	19 ± 3	42 ±5	2.5±0.2	32 ± 5	0.32 ± 0.02	5.08± 0.02
ZEA	180 ± 4a	0.74 ± 0.07a	27*±5b	55 ±4a	2.7±0.4	34 ± 3a	0.45±0.04a	5.13± 0.05a
FB_1	192* ± 4b	1.04* ± 0.07b	25 ±5a	48 ±4a	2.6±0.4	32 ± 3a	0.53*±0.04b	5.23*± 0.05a
DON	178 ± 5a	0.98* ± 0.09b	23 ±2a	47 ±3a	2.5±0.4	41* ± 4b	0.41 ± 0.02a	5.13± 0.03a
ZEA+ FB_1	190* ± 3b	1.04* ± 0.05b	25±2a	46±4a	3.2±0.4	38*± 2b	0.79*±0.06c	5.26*±0.02b
ZEA+ DON	196* ± 3b	1.13* ± 0.05c	32*±2b	65*±4b	2.5±0.4	50* ± 2c	0.42±0.06a	5.29*± 0.02b

Significantly different from the control group at the level of P <0.05. (a), (b), and (c) used to indicate the significant differences between values of animals treated. FB_1 = Fumonisin B_1, DON = deoxynivalenol, ALT = alanine aminotransferase, AST = aspartate aminotransferase, γ-GT = gamma glutamyl transpeptidase;
FB_1 = Fumonisin B_1, DON = deoxynivalenol, ZEA= zearalenone

Table 2: Unary creatinine and total proteins for female mice after oral treatment by ZEA (50 μg/kg bw/day), DON (45 μg/kg bw/day), FB_1 (110 μg/kg bw/day), and their mixtures ZEA+FB_1 and ZEA+DON; *P < 0.05 (*)*

	Creatinine (mg/dl)	**Proteins (g/dl)**
Control	86 ± 5	0.78 ± 0.02
ZEA	63*± 6a	0.84 ± 0.02
FB_1	39* ± 6b	0.82 ± 0.01
DON	86 ± 3	0.81 ± 0.015
ZEA+FB_1	25* ± 4 c	0.77 ± 0.01
ZEA+DON	32* ± 4 b	0.78 ± 0.02

(a) (b) and (c) used to indicate the significant differences between values of animals treated. FB_1 = Fumonisin B_1, DON = deoxynivalenol, ZEA= zearalenone

In blood biochemistry examinations, the significant elevation of total cholesterol and triglycerides induced by FB_1 alone or its association with ZEA revealed a plausible lipids and lipoproteins (elevation of serum proteins) metabolism disorders. These observations traduced precocious liver toxic effect induced by FB_1 but without tissue damage because no elevation of serum ALT had been found probably because of the low level of FB_1 used and/or the short duration (one week) of mice treatment in the present study. Our finding was supported by previous studies (Abbes *et al.*, 2006a; Kuiper *et al.*, 1998). Moreover, used at 50µg/kg bw/day, ZEA did not influence FB_1 toxic effect in lipids metabolism in contrast to the mixture ZEA+DON which leaded to an additive effect. Indeed, DON slightly caused an elevation of serum lipids which was more marked with its association with ZEA. In addition, the binary ZEA+DON had been found to cause an elevation of AST and ALT for females mice suggesting early liver cells lyses. But, the elevation of AST associated with rhabdomyolysis marker CK elevation as induced by DON and ZEA+DON could traduce a massive destruction of muscular tissue such as the kidney tissue (Lehmann *et al.*, 2013; Hedenmalm *et al.*, 2010). It is well established a close link renal tissue damage and increasing of serum CK concentration (Lehmann *et al.*, 2013; Hedenmalm *et al.*, 2010).

In contrast to DON, ZEA or FB_1 alone had been found to cause kidney filtration disorders as revealed by renal creatinine clearances which were twice or four times inferiors when compared to control as showed in Table 3.

Table 3: Renal clearance by serum and unary creatinine, (C_{cr} (ml/min) × 10^{-4}) for female mice after oral treatment by DON (45 µg/kg bw/day), FB_1 (110 µg/kg bw/day), and their mixture; $P < 0.05$ (*)

	Control	**ZEA**	**FB_1**	**DON**	**ZEA+FB_1**	ZEA+ **DON**
Renal clearance	22.5 ± 4.4	11 ± 2.2* a	8.43 ± 2* a	19 ± 4.1	5.16±2.3b	6±1.7*b

(a) and (b) used to indicate the significant differences between values of animals treated. FB_1 = Fumonisin B_1, DON = deoxynivalenol, ZEA= zearalenone

The mixture of FB_1 and ZEA was more potent in renal filtration disturbs as compared to individual toxin effect leading to additive effect. Similarly, our results have revealed evident disorders in renal filtration caused by both ZEA and DON. Taken together, all toxins and their mixtures exerted significant toxic effects in kidney by tissue damage or disorders in excretion or filtration functions. These results were supported by previous studies focused on FB_1, FB_2 and FB_3 preformed in rats and mice (Voss *et al.*, 1996, 1998; Gelderblom and Marasas, 2012). But macroscopic examinations were not revealed a damage of kidney of mice treated by toxins and their mixture. However, we think that a prolongation of duration of our experiments in order to show an evident damage of kidney induced by these *Fusarium* toxins tested at very low doses should lead to marked histopathological effects. In the meantime, and in order to preliminarily explore mechanism of such kidney toxicity and regarding *in vitro* high DNA methylation induced by mycotoxins ZEA, DON and FB_1 and their mixtures ZEA+DON and ZEA+FB_1 (Kouadio *et al.*, 2007), we projected to determine whether these toxins provoke DNA methylation in kidney cells *in vivo*.

Effects of Mycotoxins on DNA Methylation in Kidney Cells

The methylation of deoxycytosine (percentage of m5dC) in the DNA of kidney cells was evaluated by HPLC-UV method in the presence of individual toxin (ZEA, DON and FB_1) and their mixtures (ZEA+FB_1 or ZEA+DON). All results were summarized in Figure 2.

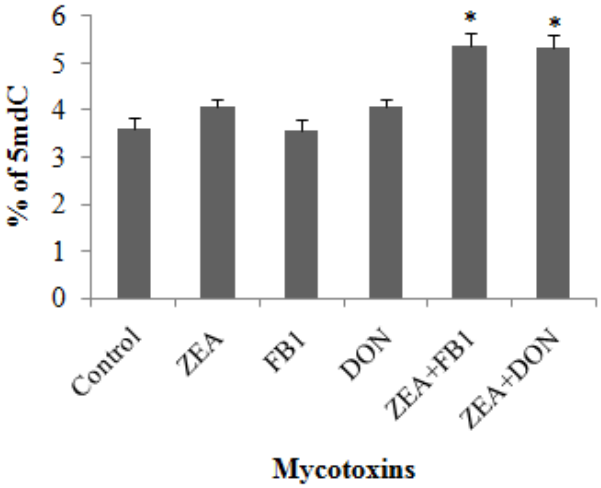

Figure 2: Deoxyribonucleic acid (DNA) methylation as measured by determination of the ratio m5dC on (dC + m5dC) × 100 in the DNA of kidney cells after oral administration of ZEA (50µg/kg bw/day), DON (45 µg/kg bw/day), FB_1 (110 µg/kg bw/day), and their mixtures (ZEA+FB_1; ZEA+DON); *P* < 0.05 (*)

ZEA or DON alone increased slightly the level of m^5dC from 3.6% to 4.07% but not FB_1. Interestingly, association of FB_1 and ZEA or DON and ZEA increased level of m^5dC until to 5.36% and 5.32% respectively. It is plausible both binaries ZEA+FB_1 and ZEA+DON leaded to an additive effect on DNA methylation in kidney cells. These findings confirmed their interactive *in vitro* effect on DNA methylation previously reported in intestinal cells line Caco-2 (Kouadio *et al.*, 2007). However, this DNA methylation in kidney cells was not linked to renal filtration because FB_1 did not impact on DNA methylation but has been found to cause renal filtration disorder in contrast to DON. Previously, it had been reported that DNA methylation elevation could be linked to radical oxygen species (ROS) produced by stress oxidative (Mobio *et al.*, 2000). In fact, the lipid, proteins or specially DNA oxidation induced by ROS could lead to elevation of DNA methylation (Mobio *et al.*, 2000; Kouadio *et al.*, 2007). In addition, the oxidation of cell membrane lipids leaded to loss of cell homeostasis maintain or to necrosis. Thus, by a high oxidative stress induced in the kidney cells, DON or ZEA and its association with FB_1 induced an elevation of serum CK which is a consequence of the destruction of the kidney tissue by necrosis (Hedenmalm *et al.*, 2010; El-Ashker, 2011).

Conclusion

The oral repetitive administration of low dose of DON and FB_1 during only 7 days has revealed disorders in lipid metabolism, renal filtration disturb and renal cell DNA methylation and rhabdomyolysis. These effects could be considering as precocious effect of both mycotoxins DON and FB_1 and their association with ZEA in female mice. On lipid metabolism and renal excretion and filtration functions, binary ZEA+FB_1 was more potent while binary ZEA+DON was more potent in liver and kidney tissues damage namely rhabdomyolysis. Taken together, our results revealed that the mixture of *Fusarium* toxins leaded to additive or more than additive effect as previous reported (Sobrova *et al.*, 2010). In addition, the no-observed effect level (NOAEL) of ZEA, DON and FB_1 were low than 50µg/kg bw/day, 45µg/kg bw/day and 110µg/kg bw/day respectively above all when they were simultaneously administrated.

REFERENCES

Abbes S, Salah-Abbes JB, Ouanes Z, Houas Z, Othman O and Bacha H (2006a). Preventive role of phyllosilicate clay on the Immunological and Biochemical toxicity of zearalenone in Balb/c mice. *International Immunopharmacology* **6** 1251-1258.

Abbes S, Ouanes Z, Ben Salah-Abbes J, Houas Z, Oueslati R and Bacha H (2006b). The protective effect of hydrated sodium calcium aluminosilicate against haemato- logical, biochemical and pathological changes induced by Zearalenone in mice. *Toxicon* **47** 567-574.

Boeira LS, Bryce JH, Stewart GG and Flannigan B (2000). The effect of combinations of Fusarium mycotoxins (deoxynivalenol, zearalenon and fumonisin B1) on growth of brewing yeasts. *Journal of Applied Microbiology* **88** 388-403.

Bondy GS, Suzuki CA, Mueller RW, Fernie SM, Armstrong CL, Hierlihy SL, Savard ME and MG Barker (1998). Gavage administration of the fungal toxin fumonisin B1 to female Sprague-Dawley rats. *Journal of Toxicology and Environmental Health* **53** 135-151.

Bucci TJ, Howard PC, Tolleson WH, Laborde JB and Hansen DK (1998). Renal effects of fumonisin mycotoxins in animals. *Toxicologic Pathology* **26** 160-164.

Collins TF, Sprando RL, Black TN, Olejnik N, Eppley RM, Alam HZ, Rorie J and Ruggles DI (2006). Effects of zearalenone on in utero development in rats. *Food and Chemical Toxicology* **44** 1455-1465.

Creppy EE (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters* **127** 19-28.

Creppy EE, Chiarappa P, Baudrimont P, Moukha S and Carratu MR (2004). Synergistic effects of fumonisin B-1 and ochratoxin A: are in vitro cytotoxicity data predictive of in vivo acute toxicity? *Toxicology* **201** 115-123.

Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS and Blunden G (2002). The occurrence of mycotoxins in key commodities in Bangladesh: Surveillance results from 1993 to 1995. *Journal of Natural Toxins* **11** 379-386.

Desjardins AE, Manandhar HK, Plattner RD, Manandhar GG, Poling SM and Maragos CM (2000). *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. *Applied and Environ-mental Microbiology* **66** 1020-1025.

Edrington TS, Kamps-Holtzapple CA, Harvey RB, Kubena LF, Elissalde MH and Rottinghaus GE (1995). Acute hepatic and renal toxicity in lambs dosed with fumonisin containing culture material. *Journal of Animal Science* **73** 508-515.

El-Ashker MR (2011). Acute kidney injury mediated by oxidative stress in Egyptian horses with exertional rhabdomyolysis. *Veterinary Research Communications* 35: 311-320.

Ennamany R, Marzetto S, Saboureau D and Creppy EE (1995). Lipid peroxidation induced by Noletu satanas: implicationin m5dC variation in Vero cells related to inhibitin of cells growth. *Cell Biology and Toxicology* **11** 347-354.

Flannery BM, Wu W and Pestka JJ (2011). Characterization of Deoxynivalenol-Induced anorexia using mouse bioassay. *Food and Chemical Toxicology* **49** 1863-1869.

Gad SC and Neil CS (1982). Statistics for toxicologists. In: *Principles and Methods of Toxicology* edited by Hayes AW (Raven Press, New York) 273.

Gajecka M and Przybylska-Gornowicz B (2012). The low doses effect of experimental zearalenone (ZEN) intoxication on the presence of Ca2+ in selected ovarian cells from pre-pubertal bitches. *Polish Journal of Veterinary Sciences* **15** 711-720.

Gelderblom WC and Marasas WF (2012). Controversies in fumonisin mycotoxicology and risk assessment. *Human & Experimental Toxicology* **31** 215-235.

Hedenmalm K, Alvan G, Ohagen P and Dahl ML (2010). Muscle toxicity with statins. Pharmacoepidemiol. *Drug Safety* **19** 223-231.

Herráez García J, Torracchi Carrasco AM, Antolí-Royo AC, De la Fuente Blanco R and Santos Jiménez MT (2012). Rhabdomyolysis. A descriptive study of 449 patients. *Medicina Clínica (Barcelona)* **139** 238-242.

Hou YJ, Zhao YY, Xiong B, Cui XS and Kim NH (2013). Mycotoxin-Containing Diet Causes Oxidative Stress in the Mouse. *PLOS ONE* **8** 6037- 6044.

Iverson F, Armstrong C, Nera E, Truelove J, Fernie S and Scott P (1995). Chronic feeding study of deoxynivalenol in B6C3F1 male and female mice. *Teratogenesis, Carcinogenesis and Mutagenesis* **15** 283-306.

Kouadio JH, Dano SD, Moukha S, Mobio TA and Creppy EE (2007). Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. *Toxicon* **49** 306-317.

Kubena LF, Edrington TS, Harvey RB, Buckley SA, Phillips TD and Rottinghaus GE (1997). Individual and combined effects of fumonisin B_1 present in Fusarium moniliforme culture material and T-2 toxin or deoxynivalenol in broiler chicks. *Poultry Science* **76** 1239-1247.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC and Safe SH (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139** 4252-4263.

Lehmann P, Hartung W and Fleck M (2013). Rhabdomyolysis and creatine kinase elevation. *Zielsetzung der ZeitschriftDie Zeitschrift für Rheumatologie* **72** 236-241.

Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, Chu I, Goddard M, Segal L, Springer JA and Myers RC (1995). Comparison of the Up-and Down, Conventional LD50, and Fixed Dose Acute Toxicity Procedures. *Food and Chemical Toxicology* **33** 223-231.

Matias WG and Creppy EE (1998). 5-Methyldeoxycytosine as a biological marker of DNA damage induced by okadaıc acidin Vero cells. *Environmental Toxicology and Water Quality* **13** 83-87.

Mobio TA, Anane R, Baudrimont I, Carratú MR, Shier TW, Dano SD and Creppy EE (2000). Epigenetic properties of fumonisin B(1): Cell cycle arrest and DNA base modification in C6 glioma cells. *Toxicology and Applied Pharmacology* **164** 91-96.

Motelin GK, Haschek WM, Ness DK, Hall WF, Harlin KS, Schaeffer DJ and Beasley VR (1994). Temporal and dose-response features in swine fed corn screenings contaminated with fumonisin mycotoxins. *Mycopathologia* **126** 27-40.

Pfohl-Leszkowicz A, Chekir-Ghedira L and Bacha H (1995). Genotoxicity of zearalenone, an oestrogenic mycotoxin: DNA adducts formation in female mouse tissues. *Carcinogenesis* **16** 2315–2320.

Ribar S, Mesaric M and Sedic M (2003). Sphingoid bases as possible diagnostic parameters. *Croatian Medical Journal* **44** 165-170.

Riley RT, Enongene E, Voss KA, Norred W, Meredith FI, Sharma RP, Spitsbergen J, Williams DE, Carlson DB and Merrill AH (2001). Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environmental Health Perspectives* **109** 301-308.

Sobrova P, Adam V, Vasatkova A, Beklova M, Zeman L and Kizek J (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology* **3** 94-99.

Tajima O, Schoen ED, Feron VJ and Groten JP (2002). Statistically designed experiments in a tiered approach to screen mixtures of Fusarium mycotoxins for possible interactions. *Food and Chemical Toxicology* **40** 685-695.

Voss KA, Riley RT, Norred WP, Bacon CW, Meredith FI and Howard PC (1996). Subchronic toxic effects of Fusarium moniliforme and fumonisin B1 in rats and mice. *Natural Toxins* **4** 16-23.

Voss KA, Plattner RD, Riley RT, Meredith FI and Norred WP (1998). *In vivo* effects of fumonisin B1-producing and fumonisin B1-nonproducing Fusarium moniliforme isolates are similar: Fumonisins B2 and B3 cause hepato- and nephrotoxicity in rats. *Mycopathologia* **141** 45-58.

Young LG, McGirr L, Valli VE, Lumsden JH and Lun A (1983). Vomitoxin in corn fed to young pigs. *Journal of Animal Science* **57** 655-664.

Zinedine A, Soriano J-M, Molto JC and Man J (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food and Chemical Toxicology* **45** 1–18.