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Subacute Toxicity Study of *A.niger* Isolates From Maize Straws in Rats

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ABSTRACT

The present study was undertaken to evaluate the toxic potential of fungal isolates from the maize straws which had caused the death of cattle in Bailhongal village in Belgaum district of Karnataka and to confirm the effects of mycotoxins produced by these isolates in rats. The fungal isolates were identified as Aspergillus niger . The fungal inoculated wheat cultures were subjected to LCMS/MS multi-mycotoxin analysis which confirmed the presence of aflatoxins and fumonisins. Sub acute toxicity study of the culture filtrates were conducted on Wistar albino rats. The animals were gavaged at three different dose levels daily for 28 days. During the study period, rats were observed for clinical signs of toxicity. Biochemical and haematological parameters also were analysed. There was a significant ($P<0.001$) increase in the serum concentrations of ALT, AST, BUN and creatinine, indicated hepatic and renal damage which got confirmed by histopathology. A significant ($P<0.001$) increase in TLC and significant ($P<0.001$) decrease in haematological parameters such as TEC, Hb and PCV was observed.

KEYWORDS : Maize straws, Toxicity, Fungi, Rats

Introduction

The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Mycotoxins are produced by some of the specific strains of filamentous fungi belonging to species of the genera *Aspergillus, Penicillium,* and *Fusarium* that invade crops at the field level and may grow on foods during storage under favorable conditions (temperature, moisture, water activity, relative humidity. The consumption of mycotoxin contaminated diet may induce acute and longterm chronic effects resulting in teratogenic, carcinogenic (mainly for liver and kidney), oestrogenic, or immunosuppressive impact not only on animals but also on man, whereas animals usually suffer more due to grains of lower quality (1)

In general, when mycotoxin-contaminated maize is mixed with the livestock feed and consumed by animals, it may result in an unhealthy situation ranging from decreased nutritive value of feed, poor feed conversion, reduced growth, organ damage and even death. The symptoms depend on the type and dosage of the mycotoxin, age, sex and species of the animal, the period for which the mycotoxin-contaminated feed is ingested and the nutritional status of the feed (4) .

Aspergillus niger is a versatile filamentous fungus found in soil, water, air, decaying plant material and large number of food and feeds all over the world (2) . In living beings , when contacted with *A. niger* and mycotoxins usually through consumption, may cause many negative effects, i.e., immunotoxcitiy, carcinogenicity and hepatotoxicity (3). (5) stated that *A.niger* has the ability to produce fumonisins the most frequently found being the fumonisin B1. and also reported the histopathologic effects of fumonisins in rats and stated that experimental models used to date to study fumonisin toxicity on laboratory animals are based mainly on the production of acute mycotoxicoses. However, consumption of lesser amounts of fumonisins at levels below those that cause overt toxicity may exert haematological, serum biochemical and/or histopathological effects in animals. (6) determined the fungi in maize, rice and millet such as *A. niger,*. and showed that these produced aflatoxin B1, fumonisin B1.

The feeding of fumonisins contaminated corn produced leukoencephalomalacia (LEM) in horses (7) and pulmonary edema (PE) and hydrothorax in swine . Experimental high doses of fumonisin caused adverse effects in cattle (8) and poultry (9). Human consumption of corn contaminated with fumonisin has been correlated with increased incidence of oesophageal cancer in certain parts of the world (10)

The objective of the present study was to Culture, isolate and identify the fungi from the affected maize straws that supposed to cause toxicity in cattle , experimentally induce mycotoxicosis in rats with fungal infected material,screen for the presence of various mycotoxins , assess changes in certain serum biochemical and hematological parameters and thereby to correlate the findings with gross and histopathological observations

Materials and Methods

Experimental site and animlas : 24 mature wistar albino rats of either sex weighing between 180+_20 g were obtained from IISc (Indian Institute of Science, Bangalore, Karnataka, India. The rats were housed in polypropylene rat cages at the Animal House of the Department of Veterinary Pharmacology and Toxicology, Veterinary college, Bangalore, Karnataka, India were the experiment was carried out. Further laboratory analysis were carried out in the Department of Veterinary Pharmacology and Toxicology, Veterinary college, Bangalore, Karnataka.This study was approved by the local ethics committee(No-147/LPM/IAEC/2013,25/09/2013).

Collection of fungal contaminated maize straws:

Fungal contaminated dry maize straws were collected from Bailhongal village of Belgaum district, where the cattle were exhibiting clinical signs of toxicity after consuming fungal contaminated maize straws like loss of body condition, ruminal atony, severe anorexia,

ataxia ,recumbency, bleeding from orifices .

Fungal isolation:
Fungal contaminated maize straw bits of 4-5 mm size were surface sterilized with 0.1% Mercuric chloride solution for 1-2 min followed by 3 succeeded washings in distilled water for 1 min each. Then the samples were inoculated into PDA Petri-plates after drying. The inoculated plates were incubated at 28^{0}C for 3-5 days+. The fungal growth on the sample was evident in about 5 days. The different fungal colonies were transferred to another plate containing PDA medium and incubated at room temperature for 5 days.

Identification of the fungi: Initial identification of the fungi was done in Dept. of Plant Pathology in University of Agricultural Sciences, Bangalore. Further confirmation was done by Agharkar Research Institute (ARI), Pune.

Brothharvesting: The pure culture was inoculated into 2 and 5 liter conical flasks containing PD broth under UV sterilized condition. The flasks were kept at 37 ^{0}C for a period of 28 days without disturbance for optimal growth of the fungi. The mycelial free content was gently decanted into another sterile container and used according to the preset protocol.

Screening: The presence of different mycotoxins was monitored using LC-MS/MS. The fungal inoculated wheat cultures were subjected to LCMS/MS multi-mycotoxin analysis which confirmed the presence of aflatoxins and fumonisins.Mycotoxin identity confirmation was performed by monitoring of at least 2 transition ions per analyte. The HPLC system utilized a binary gradient solvent system consisting of nanopure grade R0 water buffered by ammonium acetate, acetic acid and methanol. The total run time was 28 minutes per sample analyzed..

Experimentaldesign: The animals were acclimatized to the experimental laboratory conditions for a week. They were maintained under hygienic laboratory conditions providing standard laboratory feed. The animals were weighed individually at the beginning of the study and at weekly interval till day 28. The animals of each group were gavaged with the fungal culture filtrates of the respective group once daily. In the present study, the fungal culture filtrate was administered to induce the toxicity in rats since it was appropriate method to administer the desired dose of the broth culture filtrate containing major secondary metabolites or mycotoxins (30). The dose selected in rats was based on the maximum allowable dose to be administered to these animals as per the standard protocols (31). The feed intake was measured daily.The blood samples were drawn from retro-orbital plexus puncture method using microhaematocrit capillary tubes. Serum biochemical and haematological parameters were estimated on day 0, 14 and 28 .

Haematological and serum biochemical*:* Haematological parameters (erythrocyte counts, total leukocyte counts, packed cell volume (PCV), haemoglobin (Hb) concentrations and biochemical parameters (ALT,AST,creatinine , and BUN) were determined Using Semi Automatic Biochemical Analyzer- Microlab 300 (MERCK,Germany) and commercially available diagnostic kits (MERCK, Pvt, Ltd. India)

Examination of tissues: Four randomly selected rats from each treatment were killed by cervical dislocation. The sacrificed rats were carefully eviscerated to collect the organs (kidney, liver, spleen, and heart). For examination by light microscopy, the collected tissues were fixed in 10% neutral buffered formalin (pH 7.2) before dehydration in ten changes in ethanol of different concentrations ranging from 70 to 100% at 1-hr intervals. After dehydration, the tissues were cleared in two changes of chloroform before infiltration and embedding in molten wax (60°C) for 12 h. Thereafter, the tissues were blocked in paraffin wax and later sectioned using a microtome. Paraffin sections (4µm) of the tissue samples were stained with haematoxylin and eosin

Statistical analysis: Mean values and standard error of means were calculated and expressed as mean ± SEM. The data was analyzed by two-way ANOVA with Bonferroni post-tests using GraphPad Prism Trial version 5 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Table 1: 3 groups of rats of both sexes were made as follows:

Group		Dose (ml/100g) Female	No. of rats	
			Male	
Control	PD broth	2	6	6
Group IA	*Aspergillus niger* culture filtrate	0.5 (Low dose)	6	6
Group IB	*Aspergillus niger* culture filtrate	1(Medium dose)	6	6
Group IC	*Aspergillus niger* culture filtrate	2 (High dose)	6	6

RESULTS AND DISCUSSION :
Effects on haematology: The haematological indices of rats gavaged with different doses of *A.niger* fungal culture filtrate are presented in Table 2. Compared to the control group values the TLC value significantly (P<0.001) increased on 28 th day in both female and male rats. The findings in the study is supported by similar findings of many authors (11,12,13,14). This highly suggests that different doses of mycotoxins can either stimulate or suppress the immune system (15).

On day 28, TEC in female rats and male rats was significantly decreased (P<0.001) in high dose group compared to control group values. In female rats significant change (P<0.001) in Hb values were observed on 28 th day in all the 3 groups. In male rats significant (P<0.001) decrease was observed in medium dose and high dose groups on both 14 th day and 28 th day compared to control group values. PCV value was significantly (p<0.001) decreased

Table 2. 28th day Haematological indices of female rats gavaged with A.niger culture filtrate

Parameters	Control		Low dose		Medium dose		High dose	
	0 day	28 th day	0 Day	28th Day	0 Day	28th Day	0 th Day	28th Day
TLC	6.96 ±0.21	7.21±0.01	7.14 ±0.10	12.80±0.30***	6.99±0.08	11.40±0.67***	7.05±0.10	10.81±0.86***
TEC	8.03±0.35	9.09±9.09	8.30±0.09	7.790±0.22 *	8.64±0.14	7.45±0.53 **	9.01 ±0.01	6.57±0.30***
Hb	16.63 ±0.49	19.10±0.22	17.87±0.64	14.40±1.40***	16.63 ±0.47	13.43±0.13 ***	17.47±1.01	12.33±0.83 ***
PCV	45.29 ±1.39	49.93±2.53	44.05±1.58	41.10±0.66 ***	45.22 ±0.46	40.13 ±1.34 ***	47.28±0.61	39.93 ±1.24 ***

Table 3. 28th day Haematological indices of male rats gavaged with A.niger culture filtrate

Parameters	Control		Low dose		Medium dose		High dose	
	0 day	28 th day	0 Day	28th Day	0 Day	28th Day	0 th Day	28th Day
TLC	7.46±0.22	8.10±0.31	7.14 ±0.10	12.80±0.30***	6.99±0.08	11.40±0.67***	7.05±0.10	10.81±0.86***
TEC	8.78±0.25	9.40 ±0.07	8.30±0.09	7.790±0.22 *	8.64±0.14	7.45±0.53 **	9.01 ±0.01	6.57±0.30***
Hb	16.86±0.19	17.27±0.20	17.87±0.64	14.40±1.40***	16.63 ±0.47	13.43±0.13 ***	17.47±1.01	12.33±0.83 ***
PCV	47.93±0.62	49.13±0.77	44.05±1.58	41.10±0.66 ***	45.22 ±0.46	40.13 ±1.34***	47.28±0.61	39.93 ±1.24 ***

Table 4. 28 th day Serological indices of female rats gavaged with A.niger culture filtrate

Parameters	Control		Low dose		Medium dose		High dose	
	0 day	28 th day	0 Day	28th Day	0 Day	28th Day	0 th Day	28th Day
ALT	26.08±0.59	33.00±0.81	7.14 ±0.10	12.80±0.30***	6.99±0.08	11.40±0.67***	7.05±0.10	10.81±0.86***
AST	80.88±1.02	77.95±1.33	8.30±0.09	7.790±0.22 *	8.64±0.14	7.45±0.53 **	9.01 ±0.01	6.57±0.30***
BUN	25.69±2.34	23.86±0.86	17.87±0.64	14.40±1.40***	16.63 ±0.47	13.43±0.13 ***	17.47±1.01	12.33±0.83 ***
Creatinine	0.23±0.01	0.25±0.01	44.05±1.58	41.10±0.66 ***	45.22 ±0.46	40.13 ±1.34***	47.28±0.61	39.93 ±1.24 ***

Table 5: 28th day Serological indices of male rats gavaged with A.niger culture filtrate

Parameters	Control		Low dose		Medium dose		High dose	
	0 day	28 th day	0 Day	28th Day	0 Day	28th Day	0 th Day	28th Day
ALT	30.81±0.54	38.14±0.31	7.14 ±0.10	12.80±0.30***	6.99±0.08	11.40±0.67***	7.05±0.10	10.81±0.86***
AST	89.50±0.81	128.33±2.76 ***	8.30±0.09	7.790±0.22 *	8.64±0.14	7.45±0.53 **	9.01 ±0.01	6.57±0.30***
BUN	21.94±0.67	23.78±0.85	17.87±0.64	14.40±1.40***	16.63 ±0.47	13.43±0.13 ***	17.47±1.01	12.33±0.83 ***
Creatinine	0.26±0.01	0.28±0.02	44.05±1.58	41.10±0.66 ***	45.22 ±0.46	40.13 ±1.34***	47.28±0.61	39.93 ±1.24 ***

in all the 3 dose group compared to control group in female rats whereas, it was significantly (P<0.001) decreased in high dose group of male rats.

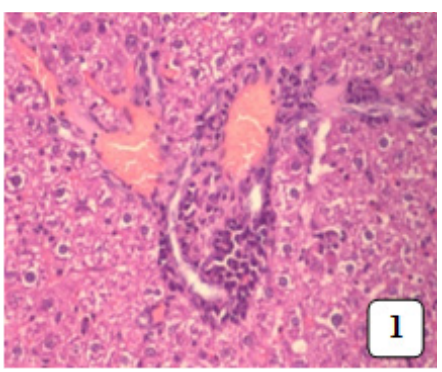
In the present study the significnat decrease in TEC, Hb and PCV were in agreement with similar findings of many authors (16,13,17) who reported that the reduction in Hb , TEC and PCV concentration observed during mycotoxicosis could be due to haemopoetic suppression and reduced protein synthesis.

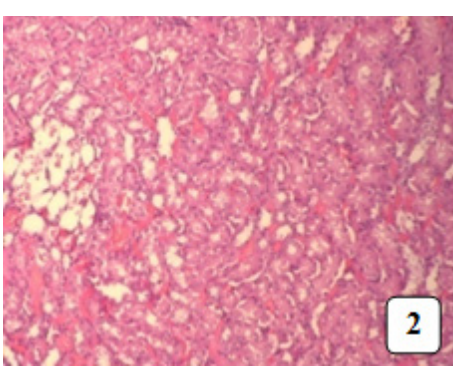
Effect on serum biochemistry:
In the present study a significant (P<0.001) increase in ALT,AST , Creatinine and BUN was observed in rats of both sexes compared to control group values.The elevated serum ALT and AST concentration in treated group compared to control group suggested the possible role of the toxins in liver and heart damage. The toxins could have altered permeability and leakage of enzyme. Other possible cause for increase in enzyme concentrations is cellular degeneration, which results in the release of intracellular enzymes into the blood stream. The results are supported by findings of many authors (18,19,20) .

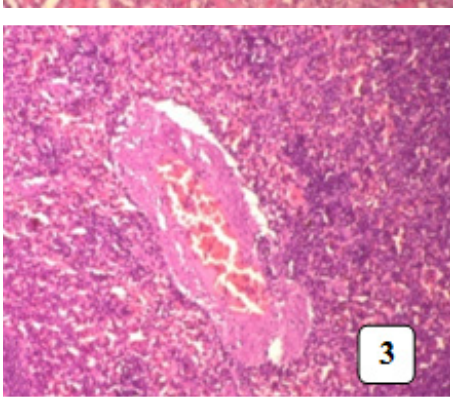
The elevated serum urea nitrogen concentration in comparison to control suggests the possible role of the toxins in causing kidney damage was also in accordance with findings of (21). The increase in creatinine values were in agreement with findings of many authors (22,23,4,24) who recorded nephrotoxicity and subsequent increase in the creatinine level in rats fed with fumonisins.

Effect on organs: Both male and female rats showed similar lesions . The lesions were more prominent in high dose groups compared to low and medium dose groups in all the organs. Microscopically lesions in the liver included severe congestion of vessels and sinusoids and haemorrhage, degeneration and necrosis of periportal hepatocytes with infiltration of inflammatory cells (Plate 1) These findings were similar to that reported by many authors (25,26,13) . The histopathological lesions of hemorrhage, congestion associated with increase in the concentrations of the serum ALT and AST enzymes reconfirmed the liver damage due to mycotoxins present in the culture filtrates of *Aspergillus niger.*

Histopathology of rats gavaged with A.niger culture filtrate







The microscopic lesions in the kidney were moderate congestion and haemorrhage, degeneration and necrosis of tubular epithelium and focal infiltration of inflammatory cells (Plate 2) was similar to the findings of (27,28) . The increased creatinine and blood urea nitrogen

concentration were also suggestive of nephrotoxic nature of the mycotoxins produced A. niger . Lymphoid hyperplasia was observed in spleen(Plate 3) which was also reported by (29) who indicated that the mycotoxins affected the lymphoid system and compensatory lymphoid hyperplasia occurred in the spleen

CONCLUSION

The rats gavaged with fungal extract containing FB1 and aflatoxin showed depressing effects on performance, biochemical and haematological parameters

indicating their adverse effects on the general health of rats . Results from this study revealed that the exposure of rats to the fumonisisn (FB1) and aflatoxin over a period of 28 days lead to leucocytosis, significant reduction in erythrocytes and haemoglobin and PCV and an increase in ALT, AST, BUN and creatinine values, and histopathological changes in some organs of rats. Fumonisins and aflatoxins have been known to occur concomitantly in fodder and the toxicological consequences of their interaction appear to be significant. The interaction between FB1 and aflatoxin for many parameters measured was significant and clearly indicates synergistic effect. Hence, it could be concluded that the present study indicated the toxic features of the fungi isolated from the infected maize straws in rats .

REFERENCES

(1)CASTEEL, S.W., ROTTINGHOUSE, G.E., (2000). Mycotoxicoses. Encycl.Microbiol., 3: 337–348. | (2) PITT, J.I. and HOCKING, A.D., (1997). Fungi and Food Spoilage 2nd ed, Blackie Academic & Professional Publishers, London | (3) MAY, G. and ADAMS, T., (1997). The Importance of fungi to Man. Genome. Res., 7: 1041-1044 | (4) NELSON, P.E., DESJARDINS, A.E. and PLATTNER, R.D., (1993). Fumonisins, mycotoxins produced by Fusarium species: Biology, chemistry and significance. Ann. Rev. Phytopathol., 31: 233-249 | (5) GELDERBLOM, W.C., JASKIEWIEZ, K., MARASAS, W.F., THIEL, P.G., HORAK, R.H. and KRIEK, N.P., (1988). Fumonisins- Novel mycotoxins with cancer- promoting activity produced by Fusarium moniliforme. Appl. Envion. Microbiol., 54: 1806- 1811 | (6) AMADI, J.E. and ADENIYI, D.O., (2009). Mycotoxin production by fungi isolated from stored Grains. Afr. J. Biotechnol., 8(7): 1219-1221 | (7)ROSS, P.F., RICE, L.G., PLATTNER, R.D., OSWEILLER, G.D., WILSON, T.M., OWENS, D.L., NELSON, H.A. and RICHARD, J.L., (1991). Concentrations of fumonisin B1 in feeds associated with animal health problems. Mycopathologia, 114(3): 129- 135 | 8) OSWEILER, G.D., KEHRI, M.E., STABEL, J.R., THRUSTON, J.R., ROSS, P.F.and WILSON, T.M., (1993). Effects of fumonisin contaminated corn screenings on growth and health of feeder calves. J. Anim. Sci., 71: 459- 466. | (9) WEIBKING, T.S., LEDOUX, D.R., BERMUDEZ, J.R.A.J., TURK, G.E., ROTTINGHAUS, G.E., WANG, E. and MERRILL, A.H.Jr., (1993a). Effects of feeding Fusarium moniliforme culture material containing known levels of fumonisin B1, on the young broiler chick. Poult. Sci., 72 (3): 456- 466. | (10) CHU, F.S. and LI, G.Y., (1994). Simultaneous occurrence of fumonisin B1 and other mycotoxins in mouldy corn collected from the people's Republic of China regions with high incidence of esophageal cancer. Appl. Environ. Microbiol., 60: 847- 852. | (11)LANZA, G.M., WASHBURN, K.W. and WYATT, R.D.,(1980). Strain variation in hematological response of broilers to dietary aflatoxin. Poult. Sci., 59: 2686-2691. | 2) BERMUDEZ, A.J., LEDOUX, D.R., TURK, J. and ROTTINGHAUS, G.E., (1996). The chronic effects of Fusarium moniniforme culture material, containing known levels of fumonisin B1 in turkeys. Avian Dis ., 40 (1): 231-5 | (13)GBORE, F.A., OLOGUNDUDU, AROWOSEGBE, A.O., OLOGUN, T.M., JOLADE, F.A. and SALIU, I., (2010). Assessment of haematological, serum biochemical and histopathological effects of subchronic dietary fumonisin B1 in rats. J. bio-sci., 18: 74-83, 2010 | (14) FAPOHUNDA, S. O., EZEKIEL, C. N., ALABI, O. A., OMOLE, A. and CHIOMA, S. O., (2008). Aflatoxin-mediated Sperm and Blood Cell Abnormalities in Mice Fed with Contaminated Corn. Mycobiology., 36(4): 255–259 | (15) MOGILNAYA, O.A., PUZYR, A.P., BARON, A.V and BONDER, V.S., (2010). Hematological parameters and the state of liver cells of rats after oral administration of aflatoxin B1 alone and together with nanodiamonds. Nanoscale. Res. Let., 5: 908-912 | (16) MOHIUDDIN, S.M., REDDY, M.V., REDDY, M.M. and RAMAKRISHNA, K., (1986). Studies on phagocytic activity and haematological changes in aflatoxicosis in poultry. Indian. Vet. J., 63: 442-445 | (17) ABDEL-WAHHAB , M.A., NADA , S.A. and KHALIL, F.A., (2002). Physiological and Toxicol ogical responses in rats fed aflatoxin contaminated diet with or without sorbent materials. Anim. Feed. Sci. Toxicol., 97(314):209 – 219 | (18) RAINA, J.S. and SINGH, B., (1991). Prevalence and pathology of mycotoxicosis in poultry in Punjab. Indian J. Anim. Sci., 61: 671-676 | (19) SAWALE, G.K., R.C. GOSH, K. RAVIKANTH, S. MAINI and.REKHE, D.S., (2009). Experimental mycotoxicosis in layer induced by ochratoxicosis A and its amelioration with herbomineral toxin binder 'Toxiroak'. Int. J .Poult. Sci., 8: 798-803 | (20) VOSS, K.A., RILEY, R. T., NORRED, W.P, BACON, C.W., MEREDITH, F.I., HOWARD P.C., PLATTNER, R.D., COLLINS, T.F., HANSEN, D.K., and PORTER, J.K., (2001). "An Overview of Rodent Toxicities: Liver and Kidney Effects of Fumonisins and Fusarium moniliforme." Environ Health Perspect., 109(2):259-66 | (21)PATHAK, B., GUPTA, S. J. and VORA, V. C., (1983). Toxicity studies of metabolites of some fungal isolates in Albino mice. Appl. Environ. Microbiol., 2: 944-947 | (22)VOSS, K.A., CHAMBERLAIN, W.J., BACON, C.W., HERBERT, R.A., WALTERS, D.B. and NORRED, W.P., (1995). Subchronic feeding study of the mycotoxin fumonisin B1 in B6C3F1 mice and Fischer 344 rats. Fundam. Appl. Toxicol., 24: 102 –110 | (23)FODOR, J., MEYER, K., RIEDLBERGER, M., BAUER, J. and POSA, R., (2006). The distribution and elimination of fumonisins after oral administration of Fusarium verticillioides fungal culture. Magy. Allatorv. Lapja., 128: 334-342 | (24) NORRED, W.P., VOSS, K.A., RILEY, R.T.and PLATTNER, R.D., (1996). Fumonisin toxicity and metabolism. Adv. Exp. Med. Biol. 392: 225 –236. | (25) ABDEL-RAHEIM, M.A., MEKI, SARY, K.H., ABDEL-GHAFFAR. and IBRAHIM EL-GIBALY., (2001). Aflatoxin B1 Induces Apoptosis in Rat Liver : Protective Effect of Melatonin. Neuroendocrinol. Lett., 22:417–426 | (26) DAFALLA, R., YAGI, A.I. and ADAM, S.E. I.,(1987). Experimental aflatoxicosis in hybro-type chicks;sequential changes in growth and serum constituents and histopathological changes. Vet. Hum. Toxicol., 29(3) : 222–225 | (27) PROZZI, C.R., CORREA, B., XAVIER, J.G., DIREITO, G.M., ORSI, R.B. and MATARAZZO, S.V., (2000). Effects of prolonged oral administratin of fumonisin B1 and aflatoxin B1 in rats. Mycopathologia., 151:21–27. | (28) HARD, G.C., HOWARD, P.C., KOVACH, R.M. and BUCCI, T.J., (2001). Rat kidney pathology induced by chronic exposure to fumonisin B1 includes rare variants of renal tubule tumor. Toxicol. Pathol., 29:379–386. | (29) TIRZIU, E., ILEANA, N., CICERONIS, C., DANIELA, M., RADU, V.G., MONICA, S. and MAGDALENA, S.M., (2011). Anatomopathological Changes Induced by Mycotoxins. Anim. Sci. Biotechnol., 44 (2):184-187 | (30)BAYMAN, P., BAKER, J.L., DOSTER, M..A., MICHAILIDES, T.J. and MAHONEY, N.E., (2002) Ochratoxin production by the Aspergillus ochraceus group and Aspergillus alliaceus. Applied Environ. Microbiol., 68(5): 2326-2329 | (31) OECD, (2001) .Organization for economic cooperation and development guidelines 403, 407, 425 and 426, for testing of chemicals. Paris, France.