Available online at
www.ijacskros.com

Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science 3 (2014) 49-60

Aflatoxins

J. Ajani[1], D.V. Sudheer Chakravarthy[1], P. Tanuja[2], K. Vali Pasha[1]*

[1]Department of Biochemistry, Yogi Vemana University, Kadapa - 516 003, Andhra Pradesh, India. [2]Department of Biochemistry, Osmania University, Hyderabad - 500 007, India.

Received 18th November2014; Revised 29th November 2014; Accepted 30th November 2014

ABSTRACT

Aflatoxin contamination of food and feed have global significance. Eventhough it is more than 50 years of aflatoxin discovery and inspite of tremendous research on various aspects of aflatoxins, it continues to be a serious challenge and health hazard to man. The adverse toxicological effects remain to be same and varies from acute effects to cancer depending on exposure. More research is necessary in developing protection strategies , biomarkers and novel technologies to combat aflatoxin contamination, keeping in mind its importance on human and animal health and in international trade.

Key words: *Aflatoxin, Biomarkers, Hepatocarcinoma, Risk reducing strategies*

1. INTRODUCTION

Aflatoxins (AFs) are a group of naturally occurring mycotoxins, which are highly carcinogenic, and consistently found as contaminants in human and animal food stuffs. AFs were first discovered in 1960 in England after the outbreak of Turkey disease that resulted in death of over 100,000 turkeys and of cancer development in rainbow trout fed on rations formulated from peanut and cotton seed meals. AFs are byproducts from the fungi *Aspergillus flavus* and the name AF was given by the virtue of its origin (*A. flavus* - *afla*). Not only *A. flavus,* but other strains of *Aspergillus* like *Aspergillus parasiticus*, *Aspergillus nomius, Aspergillus pseudotamarii, Aspergillus bombycis, Aspergillus toxicarius* and *Aspergillus parvisclerotigenus* also produce AFs. AF contamination of food and feed have gained global significance due to its deleterious effect on human and animal health and its importance in international trade and is a common problem in tropical and sub-tropical regions of the world especially in the developing countries where poor practices and environmental conditions of warm temperatures and humidity favors the growth of fungi. The occurrence of AFs in foods and food products vary with geographic location, agricultural and agranomic practices. The fungal attack occurs during preharvest, transportation, storage and processing of the foods. AFs are epidemiologically linked to increased incidence of human liver cancer in Asia and Africa [1-9].

1.2. Occurrence

Foods that are contaminated with AFs are cereals like maize, millets, sorgum, wheat and rice, oilseeds such as groundnut, sunflower, cotton and soyabean, spices like pepper, coriander, turmeric, chilies, nuts like almonds walnuts and coconuts, and milk and its products. Peanuts are susceptible products for AF contamination as per several reports [10-12]. Almonds, walnuts, and pistachios may be contaminated with AF, at lower levels than for cottonseed and corn. AF contamination of peanut is a major problem in semi-arid tropical regions. *A. flavus* infection of peanuts does not affect yield while it produces high levels of AF in infected nuts [13].

Toxins are produced on cereals, both in the field and in storage; plant [14]. Rice was significantly more heavily colonized by AF-producing *Aspergillus* spp. than other cereals, with overall AF levels being correspondingly higher. The harmful effects of such fungal invasion are discoloration of the grain and/or husk, loss in viability, loss of quality and toxin contamination. Sorghum is grown in harsh environments where other crops do not grow well. Spices are often contaminated with mycotoxins. The climatic conditions prevailing in the tropics are especially favorable for mold contamination and mycotoxin production. Clove was found to be the least contaminated while cumin was the most contaminated.

The majority of samples contained AFs at low levels and others were negative (cardamom, cloves, ginger,

*Corresponding Author:
E-mail: kotwal4pasha@yahoo.co.in,
Phone: 9849216278

and mustard) also contain AFs. Most of the AFB_1 and AFB_2 ingested by mammals are eliminated through urine and feces, however a fraction is biotransformed in the liver and excreted together with milk in the form of AFM_1 and AFM_2, respectively. AFM_1 could be detected in milk 12-24 h after the first AFB_1 ingestion, reaching a high level after a few days. Residues of AFs and their metabolites could be present in the meat, offal and eggs of animals receiving AF contaminated feeds. Poultry birds fed AF contaminated rations under experimental conditions resulted in the presence of AF residues in their edible tissues like liver and muscles. AFs are also shown to be in feeds and in animal products.

1.3. Chemistry

Of the 14 known AFs there are four key AFs: B_1, B_2, G_1 and G_2 (Figure 1). The two additional metabolic products, M_1 and M_2, are of significance as contaminants of milk, and hence the M designation. The B designation of AFs B_1 and B_2 is due to the exhibition of blue fluorescence under ultraviolet (UV)-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light. Major AFs are a group of closely related heterocyclic compounds with small differences in chemical composition.

AFB_1 and B_2, produced by *A. flavus and A. parasiticus*.

AFG_1 and G_2, produced by *A. parasiticus*.

AFM_1, metabolite of AFB_1 in humans and animals.

AFM_2, metabolite of AFB_2 in milk of cattle fed on contaminated foods.

Structural elucidation of AFB_1 was confirmed by its total synthesis [15]. Chemically, the AFs are highly substituted coumarins containing a fused dihydrofurofuran moiety. Four AFs occur naturally: B_1, B_2, G_1, and G_2. Members of the blue fluorescent (B) series are characterized by fusion of a cyclopentenone ring to the lactone ring of the coumarin moiety, whereas the green fluorescent (G) toxins contain a fused lactone ring. AFB_1 and AFG_1 possess an unsaturated bond at the 8, 9 position on the terminal furan ring, and subsequent studies demonstrated that epoxidation at this position was critical for their carcinogenic potency. AFB_2 and AFG_2 are relatively nontoxic unless they are first metabolically oxidized to AFB_1 and AFG_1 *in vivo*.

1.4. Toxicology

Over the years, outbreaks of acute aflatoxicosis in humans have been reported in regions of several economically developing countries [16]. Clinical manifestations of aflatoxicosis are vomiting, abdominal pain, pulmonary edema, and fatty infiltration and necrosis of the liver. In the 1970s, a

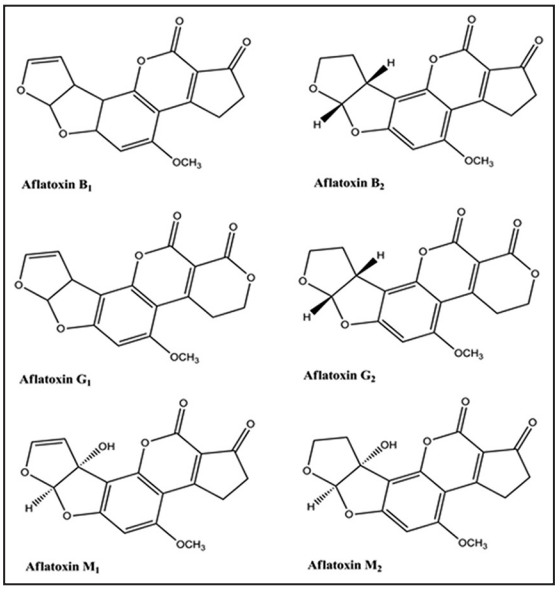

Figure 1: Aflatoxins types.

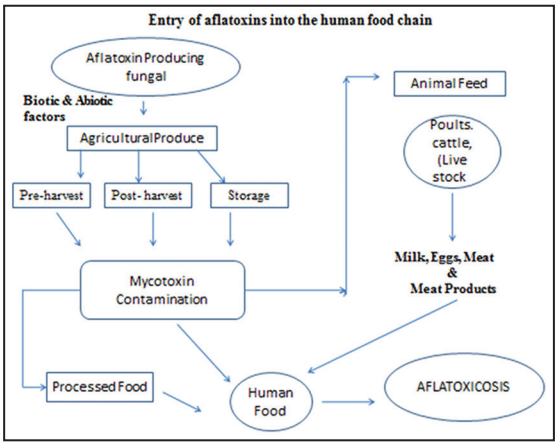

Figure 2: Entry of aflatoxins into human food chain.

putative AF poisoning in western India resulted from consumption of heavily molded corn (Figure 2). There were at least 97 fatalities and these deaths occurred only in households where the contaminated corn was eaten. Histopathology of liver specimens revealed extensive bile duct proliferation, a lesion often noted in experimental animals after acute AF exposure [17,18]. An early 1980 incident of acute aflatoxicosis in Kenya in which there were 20 hospital admissions and 20% mortality was also associated with consumption of maize highly contaminated with AF [19]. Consumption of AF-contaminated noodles resulted in acute hepatic encephalopathy in children in Malaysia; up to 3 mg of AF was suspected to be present in a single serving of contaminated noodles. Consecutive outbreaks of acute aflatoxicosis in Kenya in 2004 and 2005 caused more than 150 deaths. In April 2004, one of the largest documented aflatoxicosis outbreaks occurred in rural Kenya, resulting in 317 cases and 125 deaths. AF-contaminated maize grown and eaten on family farms was the major source of the outbreak.

Evidence of acute aflatoxicosis in human has been reported from many parts of the world, namely the third world countries like Taiwan, Uganda, India and many others. It is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma and death with cerebral edema and fatty involvement of the liver, kidneys and heart [20]. Acute aflatoxicosis results in death, whereas chronic aflatoxicosis causes cancer with the liver as the primary target organ, immune suppression, teratogenesity and symptoms [21]. There is also some evidence that respiratory exposure to AF increases the occurrence of respiratory and other cancers [2]. In a study in West Africa, majority of children tested with detectable AF levels in their blood were stunted and underweight [22]. Although AFs are known to cause cancer in animals, the U.S Food and Drug Administration (USFDA) allows them at low levels in nuts, seeds and legumes because they are considered as "unavoidable contaminants" [23].

AFs are most potent carcinogens in animal and human populations [24] and interfere with the functioning of the immune system [5]. A wide variety of animals, including fish, rodents, waterfowl, poultry, swine and cattle can be affected by AFs [3,25,26]. The International Agency for Research on Cancer (IARC) in 1993, classified naturally occurring mixtures of AFs as Class 1 human carcinogen. In fact, AFs cause liver damage, decreased milk and egg production and recurrent infection as a result of immunity suppression in addition to embryo toxicity in animals consuming low dietary concentrations. Proven signs and symptoms of aflatoxicosis in animals include gastrointestinal dysfunction, reduced, reproductivity, reduced feed utilization and efficiency, anemia and jaundice [27]. Nursing animals may be affected as a result of the conversion of AFB_1 to the metabolite AFM_1 excreted in milk of dairy cattle [28]. AFs also pose a threat to developing fetuses and they are transferred from mother to infant in breast milk.

1.5. Role of AFs in Cancer

AFs especially AFB_1, AFG_1 and AFM_1 are the most toxic, naturally occurring carcinogens known with AFB_1 the most hepatocarcinogenic compound, causing various cancers of the liver and other body organs in humans and animals [29,30]. AFs cancer causing potential is due to its ability to produce altered forms of DNA adducts. The primary disease associated with AF intake is hepatocellular carcinoma (HCC, or liver cancer). This disease is the third-leading cause of cancer death globally [30], with about 550,000-600,000 new cases each year. The incidence of liver cancer has been consistently higher in men than in women with a sex ratio ranging from 2 to 3 in most countries [6]. 83% of these cancer deaths occur in East Asia and sub-Saharan Africa [31-33]. HCC is one of the most common cancers worldwide with extremely poor prognosis. The majority of cases occur in south-east Asia and sub-Saharan Africa where the major risk factors of chronic infection with hepatitis B and C viruses as well as dietary exposure to AFs are a problem [6,30,34,35].

2. METABOLISM AND MECHANISM OF ACTION

AFs are highly liposoluble compounds and are readily absorbed from the site of exposure usually through the gastrointestinal tract (GIT) and respiratory tract into blood stream [36,37]. Human and animals get exposed to AFs by two major routes, (a) direct ingestion of AF-contaminated foods or ingestion of AFs carried over from feed into milk and milk products like cheese and powdered milk as well as other animal tissues mainly as AFM_1 [36], (b) by inhalation of dust particles of AFs especially AFB_1 in contaminated foods in industries and factories [38]. After entering the body, the AFs are absorbed across the cell membranes where they reach the blood circulation. They are distributed in blood to different tissues and to the liver, the main organ of metabolism of xenobiotics (Figure 2).

Phase I and II enzymes catalyze the conversion of a lipophilic, non-polar xenobiotic into a more water-soluble and therefore less toxic metabolite, which can then be more easily excreted from the body.

Phase II conjugation reactions which generally act follow Phase I activation consists of reactions in which metabolites containing appropriate functional groups are conjugated with substances such as glucuronate, glutamate, sulfate, reduced glutathione (GSH) or uridine diphosphate-glucuronic acid to finally discharge them through urine or bile. AFs are mainly metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful AFM_1 [4,34].

Of all the AFs, AFB_1 is the most prevalent and also the most potent. Acute dietary exposure to AFB_1 has been implicated in epidemics of acute hepatic injury [7]. The liver is the primary site of biotransformation of ingested AFB_1. The predominant human cytochrome P_{450} (CYP_{450}) isoforms involved in human metabolism of AFB_1 are CYP_{3A4} and CYP_{1A2}. In humans and susceptible animal species, AFs, especially AFB_1 are metabolized by CYP450 microsomal enzymes to AF-8,9-epoxide, a reactive form that binds to DNA and to albumin in the blood serum, forming adducts and hence causing DNA damage [4,34]. Various CYP450 enzymes isoforms occur in the liver and they metabolize AF into a reactive oxygen species (AF-8,9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer [4,34]. The predominant human CYP450 isoforms involved in human metabolism of AFB_1 are CYP3A4 and CYP1A2. Both enzymes catalyze the biotransformation of AFB_1 to the highly reactive

exo-8,9-epoxide of AFB_1 [39]. CYP 1A2 is also capable of catalyzing the epoxidation of AFB_1 to yield a high proportion of *endo*-epoxide and hydroxylation of AFB_1 to form AFM_1 which is a poor substrate for epoxidation [39] and less potent than AFB_1 [8]. This is generally considered as the major detoxification metabolic pathway for AFs. The CYP3A4 is the major CYP450 enzyme responsible for activation of AFB_1 into the epoxide form and also form AFQ_1, a less toxic detoxification metabolite. The CYP3A5 metabolizes AFB_1 mainly to the *exo*-epoxide and some AFQ_1 [40]. However, polymorphism studies with CYP3A5 have indicated that, this enzyme isoform is not expressed by most people, especially in Africans (Figure 3) [8].

2.1. Role of GSH in Detoxification of AFs and their Metabolites

GSH pathway has been shown to play a major role in the detoxification of AFB_1 [41,42]. The AFB_1 8,9 exo- and endo-epoxides can be conjugated with GSH resulting in the formation of AFB_1-mercapturate catalyzed by GSH S-transferase [41]. The exo- and endo-epoxides can also be converted non-enzymatically to AFB_1-8, 9-dihydrodiol that in turn can slowly undergo a base-catalyzed ring opening reaction to a dialdehyde phenolate ion AFB_1 dialdehyde can form Schiffs base with lysine residues in serum albumin forming AF-albumin complex [43]. Furthermore, AF dialdehyde can be reduced to a dialcohol in a nicotinamide adenine dinucleotide phosphate-dependent catalyzed reaction by AF aldehyde reductase [44,45]. The increased depletion of GSH leads to abnormally high levels of reactive oxygen species (ROS) found in cells affected by AF due to uncoupling of metabolic processes resulting from the lack of GSH for GSH-peroxidase catalysis of O_2 to H_2O_2 leading to lipid peroxidation and compromised cell membranes. Its reduction further enhances the damage to critical cellular components (DNA, lipids, proteins) by the 8,9 epoxides. However, the most serious adverse effects of the AFB_1 8,9 epoxides metabolite is that it reacts with amino acids in DNA and forms an adduct [41,42,46-52]. The increased depletion of GSH leads to abnormally high levels of ROS found in cells affected by AF due to uncoupling of metabolic processes resulting from the lack of GSH for GSH-peroxidase catalysis of O_2 to H_2O_2 leading to lipid peroxidation and compromised cell membranes. Its reduction further enhances the damage to critical cellular components (DNA, lipids, proteins) by the 8,9 epoxides. However, the most serious adverse effects of the AFB_1-8,9-epoxide metabolite is that it reacts with amino acids in DNA and forms an adduct [41,42,46-52].

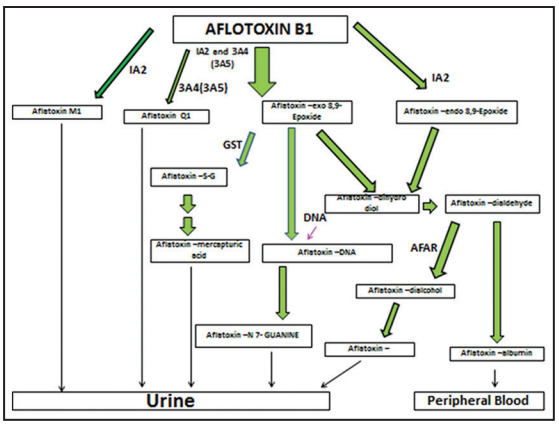

Figure 3: Metabolism of aflatoxin B_1.

2.2. Effect of AFs on Mitochondrial DNA (mitDNA) and Mitochondrial Structure

AFB causes ultrastructural changes in mitochondria [48,49] and also induces mitochondrial directed apoptosis thus reducing their function [40,53-56]. Furthermore, the AFs may affect the telomere length and the various check point in the cell cycle causing further damage to the regulatory processes of the cell cycle [56]. The reactive AF-8,9-epoxide preferentially binds to mitDNA during hepatocarcinogenesis when compared with nuclear DNA that hinder adenosine triphosphate (ATP) production and flavin adenine dinucleotide/nicotinamide adenine dinucleotide-linked enzymatic functions and this causes the disruption of mitochondrial functions in the various parts of the body that require production of energy in the form of ATP. AF damage to mitochondria can lead to mitochondrial diseases and may be responsible for aging mechanisms.

2.3. Effect of AFs on Protein Synthesis

The AF binds and interferes with enzymes and substrates that are needed in the initiation, transcription and translation processes involved in protein synthesis. They interacts with purines and purine nucleosides and impair the process of protein synthesis by forming adducts with DNA, RNA and proteins [57]. AF also inhibits RNA synthesis by interacting with the DNA-dependent RNA polymerase activity and thus causes degranulation of endoplasmic reticulum. Also, the reduction in protein content in body tissues like in skeletal muscle, heart, liver and kidney could be due to increased liver and kidney necrosis [58].

2.4. Effect of AFs on Organs and Systems

AFs have been shown to affect the various body organs such as the liver, kidneys, lungs, brain, testes and many endocrine and exocrine organs, the heart, skeletal muscles and the different body systems.

3. LIVER DISEASE

AFs have been reported to cause liver cirrhosis as well as liver cancers [4,59,60].

Hepatic injury can be acute or chronic form caused by a variety of toxic agents like AFs. The reduced level of total protein is indicative of the toxic effect

of AFB_1 to the liver due to the failure in synthesis of the proteins and kidney in which AFs are known to impair protein biosynthesis by forming adducts with DNA, RNA and proteins, inhibits RNA synthesis, DNA-dependent RNA polymerase activity and causes degranulation of endoplasmic reticulum [58,61,62]. Acute hepatic injury due to AF causes a rise in serum aspartate aminotransferase, lactate dehydrogenase, glutamate dehyrogenase, gamma-glutamyltransferase and alkaline phosphatase and bilirubin that reflect liver damage as well as other biochemical changes such as proteinura, ketonuria, glycosuria, and hematuria. It is also reported that there is a decrease in protein content in skeletal muscle, heart, liver, and kidney in AF-fed animals [58,61,62].

4. GIT AND RESPIRATORY SYSTEM

The GIT is the main route of entry of AFs as a result of consumption of AF-contaminated foods especially AFB_1. It is also the main route of excretion AF metabolites from the bile. The AFs, metabolites and AF-8,9-epoxides have been reported to cause intestinal tumors especially the human colon cancers like colon carcinomas and similar results have been reported in experimental animals [38]. AFs have also been reported to cause serious acute effects on the GIT [63]. AFs have been reported to cause digestive system effects such as diarrhea, vomiting, intestinal hemorrhage, and liver necrosis and fibrosis [64]. AFs have reported to have serious acute effects on the respiratory systems [63]. The respiratory tract is the only organ system with vital functional elements in constant and direct contact with the environment [65]. Many people working in food industries as their occupational setting get exposed to AFs, especially AFB_1 when they inhale AF-contaminated dusts like during grain shelling and processing and have been reported to have a higher incidences of upper respiratory tract and lung cancers [38,63]. AFB_1 was reported to induce 100% pulmonary adenomas in experimental animals and inhalation of AFB_1 aerosols suppressed alveolar macrophage in rats.

5. CARDIOVASCULAR AND RENAL SYSTEM

AFs have been reported to have serious acute effects on the cardiovascular systems including vascular fragility and hemorrhaging in tissues [58,63,64] as well as cardiac damage and teratogenic effects [61,62]. Exposure to AFs and its metabolites leads to nephrotoxicity [38,55]. The AF induced reduction in protein content has been reported to be due to increased necrosis of the kidney [58,61,62,66]. AFB_1 has been reported to cause kidney tumors in experimental animals and a mixture of AFB and AFG was observed to cause renal and hepatic tumors in 80% of hamsters [38]. There were also renal lesions with features of megalocytosis in the proximal renal tubules.

6. BLOOD AND IMMUNE SYSTEM

The AFs and its metabolites has been reported to have a deleterious effects on the bone and blood cells as well as induction of cancers on the hemopoietic system in bone marrow and lymphoid organs where blood, blood cells and blood components are produced [49]. AFs have been linked to anemia in pregnancy. Chronic consumption of AF-contaminated foods has been reported to cause immuno suppression in both humans and animals worldwide [7,67]. In human, AFs affect both the cellular and humoral immune responses [68-70].

7. ENDOCRINE AND REPRODUCTIVE SYSTEM

AF especially AFB has been reported to interfere with the functioning of the various endocrine gland by disrupting the enzymes and their substrates that are responsible for the synthesis of the various hormones. AFs and their metabolites as well as the generated ROS have been reported to cause various cancers in different endocrine glands such as pituitary gland, granulosa cell tumors of the ovary and adenomas and adenocarcinomas of the adrenal gland, kidneys, thyroid gland, ovaries, testes, thyroid gland, parathyroid glands, and endocrine pancreas [66,71]. In humans exposed to chronic AF higher concentrations of AFs occur in the semen of infertile men [72]. It is also associated with low birth weight, a risk factor for jaundice in infants as well as presence of AFM in maternal breast milk where it can cause deleterious effect in the newborns [67]. AFs also cause pathological alterations in the form of coagulative necrosis especially in the growing and mature follicles and decrease in number and size of graffian and growing follicles with increased number of atretic follicles and small areas of degenerative changes in experimental animals [73,74].

8. CENTRAL NERVOUS SYSTEM

AFs and its metabolites and other products such as the ROS like the AFB-8,9-epoxides may interfere with the normal functioning of the nerve cells by forming DNA adducts, protein adducts, oxidative stress factors, mitochondrial directed apoptosis of the nerve cells as well as inhibiting their synthesis of protein, RNA and DNA [44,49,51,75]. Acute AFB_1 treatment cause a decrease in regional brain acetylcholinesterase enzymes that may affect the cognitive functions as well as memory and learning of the individual while chronic exposure increases adenohypophyseal acetylcholinesterase [38]. Such deficiencies may lead to neurological symptoms such as neurocognitive decline, sleep cycle alteration, dullness, restlessness, convulsions, loss of memory, idiocy, and musle tremor [64,66].

8.1. AF Biomarkers and Risk Reducing Strategies

AF biomarkers can be used as predictors of past and future exposures status in people and establishing the

etiologic role of this toxin in human HCC and other toxicologic outcomes. Urinary measures of AFM_1, AF-mercapturic acid, and the AF-albumin adduct are used as biomarkers of internal dose. AF-N^7-guanine in urine serves as an elegant biomarker of biologically effective dose because it is clear that formation of this adduct lies on the causal pathway to AF induced HCC. Furthermore, increased formation of AF-mercapturic acid metabolites can be measured in the urine of laboratory animals as well as humans and is inversely associated with levels of AF DNA adducts formed in liver and excreted into urine [76]. These biomarkers have become critical tools for evaluation of chemopreventive agents in animal models and clinical interventions.

Postharvest intervention is one of the several methods among the various protective interventions for reducing AF risk. The use of AF biomarkers as efficacy end points in primary prevention trials has been recently reported [77]. Extensive AF exposure because of consumption of groundnuts as a dietary staple revealed that postharvest storage was correlated with increases in exposure [78,79]. The intervention comprised measures include hand sorting, drying on mats, sun drying, storage in natural fiber bags and on wooden pallets, and use of insecticides limit postharvest AF contamination of the groundnut crop [77].

Some of the trapping agents like sodium calcium aluminosilicate marketed as NovaSil clay, a common anticaking agent in animal feeds, is used to adsorb AFs in the GIT of animals and diminish the bioavailability and adverse effects of these toxins [80].

Anticarcinogenic compounds like chlorophillin and compounds like dithiolethiones that retard, block or even reverse carcinogenic process may also be employed in as detoxification strategies. Green tea polyphenols, which inhibit various chemically induced cancers may also be used to protect against the development of AFB_1 - induced hepatocarcinogenesis. Affinity chromatography using a monoclonal antibody represents a useful and rapid technique with which to isolate this carcinogen and its metabolite in biochemical epidemiology and for subsequent quantitative measurements, providing exposure information that can be used for risk assessment.

9. METHODS FOR ANALYSIS OF AFS

Currently, a range of simple to sophisticated methods are being used for the accurate analysis of AFs ranging from thin layer chromatography (TLC) to the newly developed multi-toxin liquid chromatography tandem mass spectrometry (MS) and to the rapid immunological methods [81]. Other methods to determine AFs are gas chromatography (GC), LC, TLC, high-performance liquid chromatography (HPLC), GC-MS, LCMS, immunoaffinity clean-up (IAC) coupled to HPLC and immuno assay techniques. Methods for the analysis of AFs could be categorized into, (i) biological, (ii) analytical and (iii) immunological methods. Biological assays were used when analytical and Immunological methods were not available for routine analysis. These assays are qualitative in nature and often non-specific, time consuming and less sensitive compared to those of other methods. Thus, they are often used only as a tool for screening general toxicity. Chemical tests like reaction with triflouroacetic acid or hydrochloric acid have also been developed for AF [82] with limited applications.

Various analytical methods developed for AF analysis included TLC, HPLC, high-performance thin-layer chromatography, etc. These analytical approaches for AF analysis were found to be more rapid, reproducible and possessed lower limits of detection than the biological methods [83]. Separation, detection and quantification of AFs in foods have been achieved mostly by chromatographic techniques such as TLC, HPLC and mini column chromatography. These techniques have been used for screening, presumptive or semi quantitative and quantitative purposes depending upon the limits of detection, precision and accuracy required for the analysis. In India, a Pressure mini column technique that is simple, rapid, economical, reasonably accurate and practical in field situations to detect AF has been developed and collaboratively tested [84,85]. A portable AF detection kit using dip-strip method of analysis of AFs at field level was developed as a potent research tool for public health laboratories, environmental monitoring agencies and poultry industry [86]. Nevertheless, TLC still continuous to be a major separation technique recommended to those, who cannot afford to purchase sophisticated analytical instrumentation. The published studies in which HPLC has been compared directly with TLC for the determination of AFs in peanuts [87] and maize and peanuts [88] have indicated that both techniques provide results that agree rather well. HPLC is now the most common used chromatographic technique for a detection of a wide diversity of mycotoxins, especially for AFs [59]. The analysis sample cleanup can be performed by liquid-liquid partitioning, solid phase extraction, column chromatography, IAC columns, and multifunctional clean columns. IAC combined with HPLC and fluorometric detection is used for detecting and quantifying precisely concentrations of AFM_1 [89].

However with the advent of polyclonal and monoclonal antibodies against AFs, specific enzyme-linked immunosorbent assay (ELISA) methods were developed in recent years [90] as they have been found to be simple, rapid, highly sensitive, specific and required little sample clean up. Combinations of analytical and immunological techniques such as HPLC and immunoaffinity columns have also been

used recently to screen the risk-assessment in selected areas in China [91].

Majority of the latest methods used for AF analysis are either expensive, time-consuming, involves extensive sample processing and purification and thus labor intensive. Amidst this [92] reported rather an easier and simple method involving visual estimation for AF production in groundnuts infected with *Aspergillus* mutant that accumulates an orange pigmented intermediate, norsolorinic acid [93] have earlier suggested the use of Fourier transform infrared spectroscopy as a rapid, easy and convenient method for determining AFs not only in groundnuts but also in a variety of foods and feeds. The official analytical methods for the analysis of AFs that have been validated and approved by AOAC were found to be 119 in number. The performances of each of these methods have been validated in various commodities for the analysis of total AFs/AFB_1/AFB_2/AFG_1/AFG_2/AFM_1. It was intriguing to note that 68% of these validated methods were based on HPLC, 23% on TLC, 8% on ELISA and only one method included mass spectrometry. The range of methods developed was approved based on the lower limits of detection standards developed by the European Unions for controlling AF contamination [94].

The present state of methodology for the analysis of AFs depending on the end use or purpose has been summarized. Mini column chromatography technique was found to be best for screening agricultural commodities, wherein quick decision are needed to accept or reject the produce or products. TLC, designated as veteran in AF methodology still remains a reliable, practical and simple technique with a wider field application. An alternative to TLC was found to be HPLC offering automation, separation and quantitation of AFs. However being an expensive technique it has not evolved to replace TLC. The combination of GC with MS for the analysis of AFs can provide definitive, qualitative and quantitative results, but it requires a derivatization step, which lengthens the analysis time and may compromise analyte recoveries [95]. The peak of methodical sophistication capable of detecting up to pg kg^{-1} has been achieved with GC-MS. On the other hand Immunoassays such as ELISA has been identified as the technique that will play major role for AF analysis. Besides, other alternatives will have some advantages over ELISA, as the use of magnetic droplets together with reverse transcription polymerase chain reaction, which has sensitivity to 1000 times greater than ELISA [96]. Thus analysis of AFs uses broader range of techniques who's application is largely dependent on the end use of the method.

9.1. AF and Regulations

Humans are exposed to AFs by consumption of commodities contaminated by strains of *A. flavus* or *A. paraciticus* during growth, harvest, or storage. In general, diets may contain AFB_1 and AFB_2 in concentration ratios of 1.0-0.1, and when all four AFs occur, AFB_1, AFB_2, AFG_1, and AFG_2 proportions of 1.0:0.1:0.3:0.03 exist. However, these ratios can be variable. Grains and foodstuffs contaminated with AFs include corn, peanuts, milo, sorghum, copra, and rice [9]. Although contamination by the molds may be universal, the levels or final concentrations of AFs in the grain product can vary. Heterogeneity of toxin distribution has been confirmed as the major source of error of mycotoxin determination in foods and feeds. For these reasons, estimates of human consumption of AF based on analysis of market samples of foods and foodstuffs are very imprecise [97].

Regulatory levels for AFs are in place in at least 199 countries [98,99]. The regulatory levels vary from country to country. Seventy-seven countries are known to have regulations limiting mycotoxin levels with 48 having specific regulatory levels for total AFs in foodstuffs and 21 having regulations for AFs in feedstuffs.The USFDA action levels are set at 20 ppb (μg kg^{-1}) for human food, 0.5 ppb for milk and up to 300 ppb for cottonseed cake used as animal feed. In the European Union (EU), the levels are 4.0 ppb, whereas in India the regulatory levels are set at 30 ppb for all foods [100] in 1988, IARC, Lyon, placed AFB_1 on the list of human carcinogens and identified AFB_1 in particular as a Class I carcinogen, resulting in the regulation of this toxin to very low concentrations in traded commodities 20 ppb in grains and 0.5 ppb in milk in the US; 4 ppb in foods in some European countries [101]. This is supported by a number of epidemiological studies done in Asia and Africa that have demonstrated a positive association between dietary AFs and liver cancer (Table 1) [102-104].

AF contamination also has a significant economic impact on worldwide agriculture. In the developing countries, food safety is the major problem where

Table 1: Maximum tolerated levels for AFs in human food.

Nation	Total AFs in foods (ppb)
USA	20
Australia	5
European Union	4
Germany	4
Guatemala	20
India	30
Ireland	30
Kenya	20
Taiwan	50

AF: Aflatoxin

detection and decontamination policies are impractical. Due to food shortage in those countries, routine consumption of AF contaminated food is widespread. The liver cancer incidence rates are 2-103 times higher in developing countries than in developed countries [101]. Levels ranging from 0 to 50 ppb have been set as permissible levels for AF content in foods and feeds. Most countries including the USA have a regulatory level around 20 ppb in foods. However, in 1999 European Economic Community established a lower limit of 2.0 ppb for AFB and 4.0 ppb for total AFs [105]. The AF contaminated commodities are often destroyed if the AF content is higher than the mandated levels. This results in yearly losses of billions of dollars worldwide [106]. According to FAO (2002), though 95% of the worlds peanut production is sourced from the developing countries, its contamination with AFs was attributed to environmental conditions, poor processing and lack of storage facilities in these areas. It was therefore conceived that Government agencies need to intervene to bring about market liberalization by providing incentives to adopt practices such as drying, sorting and storing. This might reduce the financial risk to market traders of peanuts [107].

AF contamination of food and feed have gained global significance due to its deleterious effect on human and animal health and its importance in the international trade. In spite of extensive research which has provided tremendous insights on various aspects of AFs, it continues to pose a serious challenge. Much remains to be elucidated and more research is necessary toward understanding of molecular mechanisms, which would improve global health and to develop protection strategies and novel technologies to combat AF contamination.

10. ACKNOWLEDGMENTS

We are highly thankful to Prof. B. Sashidhar Rao, Osmania University, Hyderabad for his help in bringing out the review.

11. REFERENCES

1. W. K. Thomas, D. R. Bill, N. W. Gerald, D. G. John, (2011) Aflatoxin: A 50 year odyssey of mechanistic and translational toxicology, ***Toxicological Sciences***, **120 Suppl 1:** S28-S48.
2. I. Dvorackova, (1990) ***Aflatoxins and Human Health***, Boca Raton, FL: CRC Press Inc.
3. J. E. Smith, M. O. Moss, (1985) ***Mycotoxins: Formation, Analysis, and Significance***, New York: Wiley, p148.
4. F. Wu, P. Khlangwiset, (2010) Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions, ***Food Additives and Contaminants,*** **27(4):** 496-509.
5. D. Hsieh, (1988) Potential human health hazards of mycotoxins. In S. Natori, K. Hashimoto, Y. Ueno, (Eds.), ***Mycotoxins and Phytotoxins,*** Elsevier, Amsterdam, The Netherlands: Third Joint Food and Agriculture Organization/ W.H.O/United Nations Environment Program, International Conference of Mycotoxins, p69-80.
6. WHO, (2000) ***Hazardous Chemicals in Humans and Environmental Health: International Programme on Chemical Safety,*** Geneva, Switzerland: World Health Organisation, p7-9.
7. D. L. Sudakin, (2003) Dietary aflatoxin exposure and chemoprevention of cancer: A clinical review, ***Journal of Toxicology- Clinical Toxicology,*** **41(2):** 195-204.
8. C. P. Wild, P. C. Turner, (2002) The toxicology of aflatoxins as a basis for public health decisions**,** ***Mutagenesis,*** **17(6):** 471-481.
9. W. F. J. Busby, and G. N. Wogan, (1984) Aflatoxins. In C. D. Searle, (Ed.), ***Chemical Carcinogens,*** Washington, DC: American Chemical Society**,** p945-1136.
10. V. Fernandez Pinto, A. Patriarca, O. Locani, G. Vaamonde, (2001) Natural co-occurrence of aflatoxin and cyclopiazonic acid in peanuts grown in Argentina, ***Food Additives and Contaminants,*** **18(11):** 1017-1020.
11. F. A. Mphande, B. A. Siame, J. E. Taylor, (2004) Fungi, aflatoxins, and cyclopiazonic acid associated with peanut retailing in Botswana, ***Journal of Food Protection*****, 67(1):** 96-102.
12. Z. Fu, X. Huang, S. Mina, (2008) Rapid determination of aflatoxins in corn and peanuts, ***Journal of Chromatography A,*** **1209(1-2):** 271-274.
13. K. V. K. Kumar, M. S. Basu, T. P. Rajendran, (2008) Mycotoxin research and mycoflora in some commercially important agricultural commodities, ***Crop Protection*****, 27(6):** 891-905.
14. C. W. Hesseltine, (1974) Natural occurrence of mycotoxin in cereals, ***Mycopathologia,*** **53(1-4):** 141-153.
15. T. Asao, G. Buichi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, G.N. Wogan, (1963) Aflatoxins B and G, ***Journal of American Chemical Society*****, 85:** 1706.
16. R. C. Shank, C. H. Bourgeois, N. Keschamras, P. Chandavimol, (1971) Aflatoxins in autopsy specimens from Thai children with an acute disease of unknown aetiology, ***Food Cosmetics Toxicology***, **9(4):** 501-507.
17. R. V. Bhat, K. A. Krishnamachari, (1977) Follow-up study of aflatoxic hepatitis in parts of western India, ***Indian Journal of Medical Research***, **66(1):** 55-58.
18. K. A. Krishnamachari, R. V. Bhat, V. Nagarajan, T. B. Tilak, (1975) Hepatitis due to aflatoxicosis. An outbreak in Western India, ***Lancet,*** **1(7915):** 1061-1063.
19. A. Ngindu, B. K. Johnson, P. R. Kenya, J. A. Ngira, D. M. Ocheng, H. Nandwa, T. N. Omondi,

A. J. Jansen, W. Ngare, J. N. Kaviti, (1982) Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya, ***Lancet,*** **12(8285):** 1346-1348.

20. T. D. Wyllie, L. G. Morchause, (1978) ***Mycotoxin Fungi, Mycotoxins, Mycotoxicoses: An Encyclopedic Handbook,*** New York: Marcel Dekker**.**
21. J. W. Bennett, M. Klich, (2003) Mycotoxins, ***Clinical Microbiological Review,*** **16:** 497-516.
22. Y. Gong, S. Egal, A. Hounsa, P.A. Turner Hall, K. Cardwell, C. Wild, (2003) Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: The critical role of weaning, ***International Journal of Epidemiology*****, 32(4):** 556-562.
23. D. L. Park, L. Stoloff, (1989) Aflatoxin control- How a regulatory agency managed risk from an unavoidable natural toxicant in food and feed, ***Regulatory Toxicology and Pharmacology*****, 9:** 109-130.
24. R. A. Squire, (1981) Ranking animal carcinogens: A proposed regulatory approach, ***Science,*** **214(4523):** 877-880.
25. R. M. Robinson, A. C. Ray, J. C. Reagor, L. A. Holland, (1982) Waterfowl mortality caused by aflatoxicosis in Texas, ***Journal of Wildlife Diseases*****, 18(3):** 311-313.
26. K. F. Higgins, R. M. Barta, R. D. Neiger, G. E. Rottinghaus, R. I. Sterry, (1922) Mycotoxin occurrence in waste field corn and ingesta of wild goose in the Northern Great Plains, ***Prairie Naturals,*** **24:** 31-37.
27. E. Talebi, M. Khademi, A. Rastad, (2011) An over review on effect of aflatoxin in animal husbandry, ***Asian Journal of Experimental Biological Sciences,*** **2:** 754-757.
28. J. H. Williams, T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. jolly, D Aggarwal, (2004) Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions, ***American Journal of Clinical Nutrition,*** **80(5):** 1106-1122.
29. D. Kitya, G. S. Bbosa, E. Mulogo, (2009) Aflatoxin levels in common foods of South Western Uganda: A risk factor to hepatocellular carcinoma, ***European Journal of Cancer Care*****, 19(4):** 516-21.
30. I. J. Beckingham, (2001) ABC of liver, pancreas and gall bladder, ***British Medical Journal,*** 322: 1-49.
31. D. D. Kirk, E. Bah, R. Montesano, (2006) Molecular epidemiology of human liver cancer: Insights into etiology, pathogenesis and prevention from The Gambia, ***Carcinogenesis,*** **27(10):** 2070-2082.
32. D. M. Parkin, (2002) Global cancer statistics, ***A Cancer Journal for Clinicians*****, 55**: 74-108.
33. H. Strosnider, (2006) Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries, ***Environmental Health Perspectives*****, 114(12):** 1989-1903.
34. C. P. Wild, R. Montesano, (2009) A model of interaction: Aflatoxins and hepatitis viruses in liver cancer aetiology and prevention, ***Cancer Letters,*** **286(1):** 22-28.
35. Z. L. Deng, Y. Ma, (1998) Aflatoxin sufferer and 53 gene mutation in hepatocelluar carcinoma, ***World Journal of Gastroenterology***, **4(1):** 28-29.
36. B. I. Agag, (2004) Mycotoxins in foods and feeds: Aflatoxins, ***Association of Universal Bulletin of Environmental Research, 7(1):*** 173-191.
37. P. Larsson, H. Tjalve, (2000) Intranasal instillation of aflatoxin B1 in rats: Bioactivation in the nasal mucosa and neuronal transport to the olfactory bulb, ***Toxicological Science*****, 55(2):** 383-391.
38. R. A. Coulombe Jr, (1994) Nonhepatic disposition and effects of aflatoxin B1. ***The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance,*** New York: Academic Press; p89-101.
39. F. P. Guengerich, (1998) Activation and detoxication of aflatoxin B1**,** ***Mutatation Research,*** **402(1-2):** 121-128.
40. H. Wang, (1998) Structure-function relationships of human liver cytochromes 450A: Aflatoxin B1 metabolism as a probe, ***Biochemistry,*** **37(36):** 12536-12545.
41. W. W. Johnson, Y. F. Ueng, M. Widersten, B. Mannervik, J. D. Hayes, P. J. Sherratt, B. Ketterer, F. P. Guengerich, (1997) Conjugation of highly reactive aflatoxin B1 exo-8, 9-epoxide catalyzed by rat and human glutathione transferases: Estimation of kinetic parameters, ***Biochemistry,*** **36(11):** 3056-3060.
42. E. O. Farombi, J. O. Nwankwo, G. O. Emerole, (2005) The effect of modulation of glutathione levels on markers for aflatoxin B1-induced cell damage, ***African Journal of Medical Sciences,*** **34(1):** 37-43.
43. G. Sabbioni, C. P. Wild, (1991) Identification of an aflatoxin G1-serum albumin adduct and its relevance to the measurement of human exposure to aflatoxins, ***Carcinogenesis,*** **12(1):** 97-103.
44. J. D. Hayes, D. J. Judah, L. I. McLellan, L. A. Kerr, S. D. Peacock, G. E. Neal, (1991) Ethoxyquin-induced resistance to aflatoxin B1 in the rat is associated with the expression of a novel alpha-class glutathione S-transferase subunit, Yc2, which possesses high catalytic activity for aflatoxin B1-8, 9-epoxide, ***Biochemical Journal*****, 279:** 385-398.
45. L. P. Knight, T. Primiano, J. D. Groopman, T. W. Kensler, T. R. Sutter, (1999) cDNA cloning, expression and activity of a second human aflatoxin B1 metabolizing member of the aldo-keto reductase superfamily, AKR7A3, ***Carcinogenesis,*** **20(7):** 1215-23.

46. P. J. Sherratt, J. D. Hayes, (2001) Glutathione S-transferase, ***Enzyme Systems that Metabolise Drugs and Other Xenobiotic,*** **9:** 320-351.
47. R. K. Stewart, C. J. Serabjit-Singh, T. E. Massey, (1996) Glutathione S-trans ferasecatalyzed conjugation of bioactivated aflatoxin B1 in rabbit lung and liver, ***Toxicology and Applied Pharmacology,*** **140(2):** 499-507.
48. D. C. Wallace, (1997) Mitochondrial DNA in aging and disease, ***Scientific American***, **277:** 40-47.
49. B. Halliwell, (2007) Oxidative stress and cancer: Have we moved forward***, Biochemistry Journal,*** **401(1):** 1-11.
50. D. Schubert, D. Piasecki, (2001) Oxidative glutamate toxicity can be a component of the excitotoxicity cascade, ***The Journal of Neuroscience,*** **21(19):** 7455-7462.
51. R. J. Verma, (2005) Aflatoxin cause DNA damage, ***International Journal of Human Genetics***, **4(4):** 231-236.
52. X. Zhang, (2005) Expression of cytochrome 450 and other biotrnasormation genes in fetal and adult human nasal mucosa, ***Drug Metabolism and Disposition,*** **33:** 1423-1428.
53. E. Jacotot, K. F. Ferri, G. Kroemer, (2000) Apoptosis and cell cycle: Distinct checkpoints with overlapping upstream control, ***Pathological Biology (Paris),*** **48(3):** 271-279.
54. K. Vermeulen, Z. N. Berneman, D. R. V. Bockstaele, (2003) Cell cycle and apoptosis, ***Cell proliferation,*** **36:** 165-175.
55. M. Peraica, (1999) Toxic effects of mycotoxins in humans, ***Bulletin of the World Health Organization,*** **77(9):** 754-766.
56. P. J. Hornsby, (2007) Senescence: As an anticancer mechanism, ***Journal of Clinical Oncology,*** **25(14):** 1852-1857.
57. J. I. Clifford, K. R. Rees, (1967) The interaction of afiatoxins with purines and purine nucleosides, ***Biochemistry Journal*****, 103(2):** 467-471.
58. V. Sharma, (2011) Ameliorative effects of curcuma longa and curcumin on aflatoxin b1 induced serological and biochemical changes in kidney of male mice, ***Asian Journal of Biochemical and Pharmaceutical Research,*** **1(2):** 338-351.
59. T. C. De Rijk, S. Arzandeh, H. C. H Kleijnen, P. Zomer, G. Van der Weg, J. G. J. Mol, (2011) Aflatoxins determination using in-line immunoaffinity chromatography in foods, ***Food Control*****, 26(1):** 42-48.
60. F. Wu, A. Tritscher, (2011) **Aflatoxins a Global Public Health Problem: Aflatoxins Health Impact,** World Health Organization. p1-18.
61. A. M. Mohammed, N. S. Metwally, (2009) Antiaflatoxicogenic activities of some aqeous plant extracts against AFB1 induced renal and cardiac damage, ***Journal of Pharmacology and Toxicology*****, 4(1):** 1-16.
62. P. B. Wangikar, (2005) Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and aflatoxin B1 with special reference to microscopic effects, ***Toxicology,*** **215(1-2):** 37-47.
63. N. Gursoy, (2008) Changes in spontaneous contractions of rat ileum by aflatoxin *in vitro*, ***Food Chemistry and Toxicology,*** **46(6):** 2124-2127.
64. A. M. Harriet, (2003) Is indoor mold contamination a threat to health? ***Journal of Environmental Health***, **62(2):** 0022-0892.
65. A. Kussak, B. Andersson, K. Andersson, (1995) Determination of aflatoxins in air borne dust from feed factories by automated immunoaffinity column clean-up and liquid chromatography, ***Journal of Chromatography A.,*** **708(1*):*** 55-60.
66. A. W. Lakkawar, S. K. Chattopadhyay, T. S. Johri, (2004) Experimental aflatoxin B1 toxicosis in young rabbits - A clinical and patho-anatomical study, ***Slovenian Veterinary Research,*** **41:** 73-81.
67. F. M. B. Shuaib, (2010) Association between anemia and aflatoxin B1 biomarker levels among pregnant women in Kumasi, Ghana, ***American Journal of Tropical Medicine and Hygiene,*** **83(5):** 1077-1083.
68. Y. Jiang, (2005) Aflatoxin B1 albumin adduct levels and cellular immune status, ***Ghanaians International Immunology,*** **17(6):** 807-814.
69. Y. Jiang, (2008) Aflatoxin-related immune dysfunction in health and in human immunodeficiency virus disease, ***Clinical Developmental Immunology***, **2008:** 1-12.
70. P. K. Sahoo, S. K. Chattopadhyay, A. Sikdar, (1996) Immunosuppressive effects of induced aflatoxicosis in rabbits, ***Journal of Applied Animal Research*****, 9:** 17-26.
71. K. Goerttler, (1980) Effects of aflatoxin B1 on pregnant inbred sprague-dawley rats and their F1 generation, a contribution to transplacental carcinogenesis 1, 2, 3***, JNCI Journal of the Natational Cancer Institute,*** **64(6):** 1349-1354.
72. R. C. Gupta, (2011). Aflatoxins. Ochratoxins and citrinins, ***Reproductive and Developmental Toxicology,*** **55:** 753-761.
73. S. M. El-Azab, (2009) Study of aflatoxin B1 as a risk factor that impair the reproductive performance in females - Egypt, ***The Internet Journal of Toxicology,*** **6(1):** 240-265.
74. K. L. Brown, (2009) Inherent stereospecificity in the reaction of aflatoxin B18,9-epoxide with deoxyguanosine and efficiency of DNA catalysis, ***Chemical Research in Toxicology,*** **22(5),** 913-917.
75. C. N. Ezekiel, (2011) Studies on dietary aflatoxin-induced genotoxicity using two *in vivo* bioassays, ***Archives of Applied Science Research,*** **3(2):** 97-106.
76. J. S. Wang, X. Shen, X. He, Y. R. Zhu, B. C. Zhang, J. B. Wang, G. S. Qian, S. Y. Kuang, A. Zarba, P. A. Egner, L. P. Jacobson, (1999) Protective alterations in phase 1 and 2 metabolism

of aflatoxin B1 by oltipraz in residents of Qidong, people's republic of China, ***Journal of National Cancer Institute,*** **91(4):** 347-354.
77. P. C. Turner, A. Sylla, Y. Y. Gong, M. S. Diallo, A. E. Sutcliffe, A. J. Hall C. P. Wild, (2005) Reduction in exposure to carcinogenic aflatoxins by postharvest intervention measures in West Africa: A community-based intervention study, ***Lancet,*** **365(9475):** 1950-1956.
78. M. S. Diallo, A. Sylla, K. Sidibe, B. S. Sylla, C. R. Trepo, and C. P Wild, (1995) Prevalence of exposure to aflatoxin and hepatitis B and C viruses in Guinea, West Africa. ***Natural Toxins,*** **3(1):** 6-9.
79. A. Sylla, M. S. Diallo, J. Castegnaro, C. P. Wild, (1999) Interactions between hepatitis B virus infection and exposure to aflatoxins in the development of hepatocellular carcinoma: A molecular epidemiological approach, ***Mutaion Research,*** **428(1-2):** 187-196.
80. T. D. Phillips, S. L. Lemke, P. G. Grant, (2002) Characterization of claybased enterosorbents for the prevention of aflatoxicosis, ***Advances in Experimental Medicine and Biology,*** **504:** 157-171.
81. G. G. Shepard, (2009) Aflatoxin analysis at the beginning of the twenty-first century, ***Analytical Bioanalytical Chemistry,*** **395(5):** 1215-1224.
82. S. Nesheim, W. C. Brumley, (1981) Confirmation of identity of aflatoxins, ***Journal of American oil Chemists Society*****, 64:** 945A-949A.
83. H. P. Van Egmond, W. E. Paulsch, (1986) Mycotoxins in milk and milk products, ***Netherlands Milk and Dairy Journal*****, 40:** 175-188.
84. R. B. Sashidhar, B. S. Narasinga Rao, (1988) Non-competitive enzyme linked immunosorbent assay for the detection of aflatoxin B_1, ***Indian Journal of Experimental Biology*****, 26(12):** 984-989.
85. R. B. Sashidhar, R. V. Sudershan, Y. Ramakrishna, S. Nahdi, R. V. Bhat, (1989) Enhanced fluorescence of ergosterol by iodination and determination of ergosterol by fluorodensitometry, ***Analyst*****, 113(5):** 809-812.
86. R. B. Sashidhar, (1993b) Dip-strip method for monitoring environmental contamination of aflatoxin in food and feed: Use of a portable aflatoxin detection kit, ***Environmental Health Perspectives*****, 101 Suppl 3:** 43-46.
87. D. C. Hunt, A. T. Bourdon, P. J. Wild, N. T. Crosby, (1978) Use of high performance liquid chromatography combined with fluorescence detection for the identification and estimation of aflatoxins and ochratoxin in food, ***Journal of the Science of Food and Agriculture*****, 29(3):** 234-238.
88. J. W. De Vries, H. L. Chang, (1982) Comparison of rapid high pressure liquid chromatographic and CB methods for the determination of aflatoxins in corn and peanuts, ***Journal Association of Official Analytical Chemist,*** **65(2):** 206-209.
89. J. E. Lee, B. M. Kwak, J. H. Ahn, T. H. Jeon, (2009) Occurrence of aflatoxin M1 in raw milk in South Korea using an immnoaffinity column and liquid chromatography, ***Food Control,*** **20(2):** 136-138.
90. J. J. Pestka, P. K. Gaur, F. S. Chu, (1980) Quantitation of aflatoxin B1 and B1 antibody by an enzyme linked immunosorbent microassay, ***Applied and Environmental Microbiology*****, 40(6):** 1027-1031.
91. X. Ding, P. Li, Y. Bai, H. Zhou, (2012) Aflatoxin B1 in post-harvest peanuts and dietary risk in China, ***Food Control*****, 23:** 143-148.
92. Y. Lopez, N. P. Keller, B. Sarr, T. D. Phillips, R. G. Cuero, O. D. Smith, (1998) Visual estimation of aflatoxin production in peanut with *Aspergillus* norosolorinic acid mutants, ***Peanut Science*****, 25:** 92-99.
93. M. E. S. Mirghani, Y. B. Che Man, S. Jinap, B. S. Baharin, J. Bakar, (2001) A new method for determining aflatoxins in groundnut and groundnut cake using fourier transform infrared spectroscopy with attenuated total reflectance, ***Journal of the American Oil Chemists' Society,*** **78:** 985-992.
94. J. Gilbert, E. Anklam, (2002) Validation of analytical methods for determining mycotoxins in foodstuffs, ***Trends in Analytical Chemistry,*** **21:** 468-486.
95. H. Wang, Z. Xiangjuan, Y. Liu, H. Yang, Q. Guo, (2011) Simultaneous determination of chloramphenicol and aflatoxin M1 residues in milk by triple quadrupole liquid chromatography-tandem mass spectrometry, ***Journal of Agricultural and Food Chemistry*****, 59(8):** 3532-3538.
96. D. Babu, P. M. Muriana, (2011) Immunomagnetic bead-based recovery and real time quantitative PCR (RT iq-PCR) for sensitive quantification of aflatoxin B(1), ***Journal Microbiological Methods,*** **86(2):** 188-194.
97. J. L. Richard, G. A. Bennett, P. F. Ross, P. E. Nelson, (1993) Analysis of naturally occurring mycotoxins in feedstuffs and food, ***Journal of Animal Science,*** **71(9):** 2563-2574.
98. H. P. Van Egmond, M. A. Jonker, (2005) Worldwide regulations on aflatoxins. In H.K. Abbas (ed.) ***Aflatoxin and Food Safety,*** Boca Raton, FL: Taylor and Francis, p77-93.
99. F. Wu, (2010) Mycotoxin risk assessment for the purpose of setting international regulatory standards, ***Environmental Science and Technology***, **38(15):** 4049-4055.
100. M. A. Klich, (2007) *Aspergillus flavus*: The major producer of aflatoxin, ***Molecular Plant Pathology,*** **8(6):** 713-722.
101. S. H. Henry, F. X. Bosch, T. C. Troxell, P. M. Bolger, (1999) Reducing liver cancer-global

control of aflatoxin, ***Science,* 286:** 2453-2454.
102. Y. P. Dragon, H. C. Pitot, (1993) Aflatoxin carcinogenesis in the context of the multistage nature of cancer. In D. L Eaton, J. D. Groopman (Eds.), ***The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance,*** London: Academic Press, p179-206.
103. N. J. Gorelick, R. D. Bruce, M. S. Hoseyni, (1993) Human risk assessment based on animal data: Inconsistencies and alternatives. In D. Eaton, J. D Groopman, (Eds.), ***The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance***, London: Academic Press, p508-511.
104. J. D. Kelly, D. L. Easton, F. P. Guengerich, R. J. Coulombe, (1997) Aflatoxin B sub (1) activation in human lung, ***Toxicolology and Applied Pharmacology,* 144:** 88-95.
105. N. H. Mishra, D. Chitrangada, (2003) A review on biological control and metabolism of aflatoxin, ***Critical Reviews in Food Science and Nutrition*, 43(3):** 245-264.
106. J. F. Robenson and K. Cardwell, (2005) The cost of mycotoxin management in the United States, In H. K Abbas, (Ed.), ***Aflatoxin and Food Safety,*** Boca Ration, FL: CRC Press, p1-12.
107. C. B. N'dede, C. M. Jolly, S. D. Vodouhe, J. E. Jolly, (2012) Economic risks of aflatoxin contamination in marketing of peanut in Benin, ***Economics Research International*, 2012:** 1-12.

****Bibliographical Sketch***

Dr. K. Vali Pasha has done his M.Sc and Ph.D from University of Hyderabad, Hyderabd in the years of 1982 and 1988 respectively. His major areas of research are neurobiochemistry, clinical biochemistry, herbal drugs, antioxidants. He was a NIH post-doctoral fellow at University of Connecticut, USA. He was a faculty member at Jamia Hamdard University, New Delhi and later moved to Nizam Institute of Medical Science,Hyderabad Where he worked as Asst.Professor and Associate Professor. At present he is a Professor of Biochemistry and Dean of Faculty of Science in Yogi Vemana University, Kadapa,Andhra Pradesh, India.