Review of Literature
Aflatoxins are the toxic secondary metabolites produced by fungi on food and feed materials. Some of these cause severe effects on animals, plants and microbial systems. Aflatoxin B1 (AFB1) is the predominant toxin of all the aflatoxins (Guan et al, 2008). Several attempts have been made to detoxify aflatoxin contaminated foods and feeds by using physical, chemical and biological methods. The present study is an attempt to ameliorate the aflatoxin B1 toxicity using selected plant products. The review of literature pertaining to the study “Protective effect of Moringa oleifera and Aloe vera on aflatoxin B1 induced toxicity” is discussed under the following headings:

2.1 AFLATOXINS
   2.1.1 OCCURRENCE AND SYNTHESIS
   2.1.2 TYPES AND STRUCTURE
   2.1.3 EXPOSURE AND ABSORPTION

2.2 METABOLISM OF AFLATOXIN B1

2.3 AFLATOXIN RESIDUES IN ANIMAL PRODUCTS

2.4 EFFECT OF AFLATOXINS ON HUMANS
   2.4.1 EFFECT OF ACUTE EXPOSURE TO AFLATOXINS IN HUMANS
   2.4.2 EFFECT OF CHRONIC EXPOSURE TO AFLATOXINS IN HUMANS

2.5 EFFECT OF AFLATOXINS ON POULTRY

2.6 ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT OF AFLATOXIN INDUCED TOXICITY
   2.6.1 FREE RADICALS AND OXIDATIVE STRESS
   2.6.2 LIPID PEROXIDATION

2.7 ANTIOXIDANT DEFENSE IN COMBATING OXIDATIVE STRESS
2.8 STRATEGIES TO CONTROL AND DETOXIFY AFLATOXIN

2.8.1 GOOD AGRICULTURAL AND MANUFACTURING PRACTICES
2.8.2 DECONTAMINATION METHODS
2.8.3 CHEMOPREVENTION TO COUNTERACT AFLATOXIN TOXICITY

2.9. Moringa oleifera

2.9.1 MORPHOLOGY OF Moringa oleifera
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2.10. Aloe vera

2.10.1 MORPHOLOGY OF Aloe vera
2.10.2 BIOLOGICAL IMPORTANCE OF Aloe vera

2.11. PROTECTIVE ROLE OF Silymarin in AFB1 TOXICITY

2.1 AFLATOXINS

Aflatoxins are a group of closely related heterocyclic compounds, first discovered in 1960 in England after the outbreak of “Turkey-X-disease” when 1,00,000 turkey poults died in England (Blount, 1961) and named as an abbreviation of “Aspergillus flavus toxin” - Aflatoxins (Nesbit et al., 1962).

2.1.1 OCCURRENCE AND SYNTHESIS

Aflatoxins are produced in a vast array of dietary staples and agricultural products such as rice, corn, cassava, peanuts, cottonseed, pistachios, copra and figs with widespread contamination in hot and humid regions of the world (Farombi, 2006; Murphy et al., 2006).
Aflatoxins are produced predominantly by the toxic species of Aspergillus fungi namely *Aspergillus parasiticus* (Plate 1) and *Aspergillus flavus* (Plate 2) as secondary metabolites (Toratora *et al.*, 2008). An optimum temperature in the range of 24°C – 35°C and moisture content above seven percent were recorded to be favourable for the fungal growth and aflatoxin production (Williams *et al.*, 2004). The toxin production was found to be dependent on the genotype of the crop planted, soil type (Brown, *et al.*, 2001; Bankole and Mabekoje 2004), stress or damage to the crop due to drought prior to harvest, insect activity, poor timing of harvest, heavy rains at harvest and post-harvest and inadequate drying of the crop before storage (Hell *et al.*, 2000; Onoci *et al.*, 2002; Hawkins *et al.*, 2005; Turner *et al.*, 2005).

### 2.1.2 TYPES AND STRUCTURE

Chemically, aflatoxins are a group of difuranocoumarin derivatives. Based on the colour of the fluorescence under ultraviolet light, blue or green, the aflatoxins are grouped into aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), and aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). The numbers 1 and 2 refer to the chromatographic mobility. Aflatoxins M1 and M2 are the milk-aflatoxins and the metabolites of AFB1 and AFB2 respectively. Among the aflatoxins, AFB1 was identified to be the most toxic and most prevalent compound, followed by G1, B2 and G2 with decreasing toxicity (Murphy *et al.*, 2006).

The chemical structures of major dietary aflatoxins namely aflatoxins B1, G1 and M1 with the double bonds and aflatoxins B2, G2 and M2 without the double bond in 8-9 positions are shown in Figure 1.
2.1.3 EXPOSURE AND ABSORPTION

Aflatoxin B1 is a common food and feed contaminant. Exposure in humans and animals mainly occurs through the oral route. However, in people handling contaminated grains, inhalation of contaminated grain dust was
reported to be a major source of AFB1 exposure (Desai and Ghosh, 2003). Since AFB1 is a relatively low molecular weight compound, after ingestion, aflatoxins are efficiently absorbed in the intestinal tract probably by passive diffusion. From the intestine AFB1 apparently enters liver through the hepatic portal blood supply (Gratz et al., 2007). It was suggested that no efflux pumps or transporters were involved in AFB1 absorption or extrusion (Mata et al., 2004). It has been demonstrated by Coulombe and Sharma (1985) that after 4 hours of exposure, the plasma concentration – time plots did not differ between oral and respiratory routes of exposure.

2.2 METABOLISM OF AFLATOXIN B1

Aflatoxin B1 is not a potent toxin by itself. Bioactivation is needed to exert its toxic effects. The predominant human cytochrome P450 (CYP450) involved in human metabolism of AFB1 are CYP3A4 and CYP1A2. (Guengerich, 2001). Both catalyze the biotransformation of AFB1 to the highly reactive exo-8,9-epoxide of AFB1 (Smela et al., 2001). CYP1A2 also catalyzes the epoxidation of AFB1 to yield a high proportion of endo epoxide and hydroxylation of AFB1 to aflatoxin M1 (AFM1). AFM1 is a less potent metabolite than AFB1 and is considered as a detoxification metabolite of AFB1 (Wild and Turner, 2002).

A majority of enzyme action of CYP3A4 catalyzes the hydroxylation AFB1 to AFQ1, a less toxic detoxification metabolite. CYP 3A5 metabolizes AFB1 mainly to the exo epoxide and some AFQ1 (Farombi, 2006). Among the two isomers, the endo-and exo-form of AFB1-8, 9-epoxide, only the exo-isomer is of relevance in terms of toxicity and carcinogenicity (Guengerich, 2001). The biotransformation pathways for AFB1 are given in Figure 2.
FIGURE 2
OVERVIEW OF BIOTRANSFORMATION PATHWAYS FOR AFlATOXIN B1
(Bammler et al., 2000)

Aflatoxin B1

Cytochrome P450

Hydroxylated Metabolites: AFM1, AFQ, AFP1

DNA repair

DNA adducts

Mutation

Cancer

Aflatoxin B1-8,9-epoxide (exo and endo)

Glutathione S-transferase

Aflatoxin B1-glutathione conjugate

Excretion

Physiological pH

Dialdehydic phenolate

Aflatoxin B1 dialcohol

Excretion

Aflatoxin B1 aldehyde reductase

Protein binding

Toxicity
The AFB1 - 8,9-exo and endo epoxides are detoxified by conjugating with glutathione, resulting in the formation of the nontoxic, aflatoxin B-mercapturic acid. The reaction is catalyzed by glutathione-S-transferase (GST), an enzyme essential in the reduction and prevention of AFB1 induced carcinogenicity (Wild and Turner, 2001). The exo and endo epoxide can also be converted non enzymatically to AFB1-8, 9-dihydrodiol, which in turn can slowly undergo a base-catalyzed ring opening reaction to a dialdehyde phenolate ion. This can be reduced to a dialcohol in an NADPH – dependent reaction catalyzed by aflatoxin aldehyde reductase (AFAR) and the dialcohol was found to be excreted (Farombi, 2006). The enzymes GST and AFAR were identified as being uniquely important in the detoxification of AFB1 in rats (Kimura et al., 2004).

Reduction of the 1-keto group of AFB1 produces the metabolite aflatoxicol (AFL) and the reaction is catalyzed by a cytosolic reductase. AFL on the other hand can be readily oxidized back to AFB1 and was suggested as a “reservoir” for AFB1 in vivo (Gratz et al., 2007).

Aflatoxin and its metabolites are excreted in bile, urine, faeces and milk. The hydroxylated AFB1 metabolites AFM1, AFQ1, demethylation metabolites AFQ1 and AFB1-N7-guanine, the degradation product of hepatic AFB1-DNA adducts were found to be excreted in urine of rats and humans (Egner et al., 2003; Kensler et al., 2005). About 50% of AFB1 metabolites from the liver were excreted in the feces via the bile mainly as AFB1-glutathione conjugate (IARC, 2002). Mykkanen et al. (2005) reported a 10-fold higher level of AFQ1 in urine compared to that of AFM1 and AFQ1 and AFM1 in faeces of AFB1 exposed subjects. In poultry, aflatoxin and its metabolites were excreted mainly through bile and to a lesser extent through the kidney and gastro intestinal tract (Leeson et al., 1995).
2.3 AFLATOXIN RESIDUES IN ANIMAL PRODUCTS

Some of the metabolites formed during the metabolism of AFB1 may be transmitted to edible animal products. Aflatoxins present in contaminated feed tend to infiltrate liver, muscle, kidney and gizzard of the chicken (Bintvihok and Davitiyananda, 2002; Fernandez et al., 2006). Furthermore, aflatoxin and its metabolites were also suggested to be transferred to eggs in laying hens (Oliveira et al., 2000; Sur and Celik, 2003). The transmission ratio of AFB1 from layer food into the egg was reported to be 1/2000 to 1/2500 (Oliveira et al., 2000). Consumption of such contaminated products could lead to cancer and thus pose a high risk to human health (Bintvihok and Davitiyananda, 2002).

2.4 EFFECT OF AFLATOXINS ON HUMANS

2.4.1 EFFECT OF ACUTE EXPOSURE TO AFLATOXINS IN HUMANS

Outbreaks of acute aflatoxin poisoning have been a recurrent public health problem. Acute exposure to aflatoxin was found to result in direct liver damage and subsequent illness or death (Williams et al., 2004). In India, in 1974, an outbreak of hepatitis was reported in 200 villages in Rajasthan and Gujarat. The consumption of maize samples contaminated with 6.25 - 156 ppm aflatoxins for one month was found to be responsible for 106 deaths. A correlation between aflatoxin contamination and hepatomegaly in children was reported in South Canara district of Karnataka (Reddy and Raghavender, 2007).

2.4.2 EFFECT OF CHRONIC EXPOSURE TO AFLATOXINS IN HUMANS

For humans, aflatoxin is predominantly perceived as an agent promoting liver cancer (Guan et al., 2008) although respiratory exposure has been reported to cause lung cancer (Yang et al., 2006). AFB1-8,9-exo epoxide formed during the metabolism of AFB1, binds with high affinity to guanine bases in DNA to form aflatoxin-N guanine which is responsible for its mutagenic and
carcinogenic effects (Bedard and Massey, 2006). Aflatoxins –N guanine formed guanine (purine) to thymine (pyrimidine) transversion mutations in DNA at codon 249 of the P53 tumor suppressor gene (Eaton and Gallagher, 2004). The G to T transversion mutations induced by AFB1-8, 9-epoxide at codon 249 of P53 gene has been found with greater frequency among patients with hepatocellular carcinomas in areas of high risk aflatoxin exposure (Farombi, 2006).

Exposure to aflatoxin has been associated with immunosuppression and growth retardation in humans (Turner et al., 2003; Gong et al., 2002). Chronic AFB1 exposure also seemed to exacerbate protein calorie malnutrition (kwashiorkor) although it has not been shown to be responsible for the development of the condition (Gong et al., 2004).

2.5 EFFECT OF AFLATOXINS ON POULTRY

The first outbreak of aflatoxin poisoning (aflatoxicosis) in India was reported in 1966 at Government Poultry Breeding unit, Hebbal, Bangalore, Karnataka when 2219 chicks died. In 1982, heavy mortality in chicks occurred in Chittoor district of Andhra Pradesh due to aflatoxicosis, caused by ingestion of aflatoxin contaminated groundnut cake. In 1985, an aflatoxicosis outbreak in poultry caused 85% to 40% drop in egg- production in Warangal, Andhra Pradesh. In 1994, more than 200,000 broiler chickens died in Ranga Reddy district of Andhra Pradesh after consuming aflatoxin contaminated ground nut cake in feeds (ICRISAT, 2002 ; Reddy and Raghavender, 2007).

Aflatoxin poisoning in poultry is characterized by weakness, anorexia with lowered growth rate, poor feed utilization, decreased weight gain, decreased egg production, liver tumors, increased susceptibility to environmental and microbial stresses and increased mortality (Tedesco et al., 2004). Low levels of aflatoxin seemed to affect the cell mediated immunity while high levels affected humoral immunity (Oguz et al., 2003).
Aflatoxin toxicity in poultry was also associated with biochemical, hematological (Basmaciaglu et al., 2005), reproductive (Ortatatlì et al., 2002) and pathologic changes (Ortatatlì et al., 2005). The aflatoxin toxicity in broiler chickens has been widely investigated by carcinogenic, mutagenic and teratogenic effects (Wild et al., 2000; Sur and Celik, 2003).

The lethal dose for 50% of animals (LD$_{50}$) for poultry was 6.5 to 16.5mg/kg, for rats of age 21 days was 5.5 and for rats weighing 100gm was 17.9. The LD$_{50}$ values of animals depend on age, sex, species, strain, duration of exposure, nutrition, health of animals and drugs (Agag, 2004).

2.6 ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT OF AFLATOXIN INDUCED TOXICITY

2.6.1 FREE RADICALS AND OXIDATIVE STRESS

Free radicals are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. They are produced during the normal metabolism of cells. Many types of radicals exist but those of most concern in biological systems are derived from oxygen and are referred as reactive oxygen species (ROS). The sequential reduction of molecular oxygen leads to the formation of a group of reactive oxygen species superoxide anion (O$_2^-$) hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH) and singlet oxygen ($^1$O$_2$), eventually terminating in the formation of water (Eraslan et al., 2004a; Guven and Kaya, 2005). The uncoupled electrons of free radicals are very reactive with adjacent molecules such as lipids, proteins and carbohydrates and were suggested to cause cellular damage (Halliwell, 2000). As a result of the relative instability of free radicals and their potential to damage cells and tissues, there are both enzymes and small molecular-weight molecules with antioxidant capabilities that can protect against the adverse effects of free radical reactions (Guven and Kaya, 2005). Exposure to chemicals and other stress conditions increases the production of ROS. This results in disturbance in
the prooxidant – antioxidant balance in favour of the former, leading to a condition termed “oxidative stress” (Kurien and Scofield, 2006). Aflatoxins were demonstrated to induce the production of ROS and oxidative stress has been suggested as one of the underlying mechanisms for AFB1 induced cell injury and DNA damage (Yang et al., 2000b; Surai, 2002).

### 2.6.2 LIPID PEROXIDATION

Lipids are the major target for free radical attack in part because oxygen is more soluble in hydrophobic membrane. The free radicals react with biomembrane causing oxidative destruction of polyunsaturated fatty acids (PUFA) forming autotoxic aldehydes such as malondialdehyde (MDA) and other related carbonyl compounds by a process known as lipid peroxidation. Lipid peroxidation (LPO) severely damages the cell lipid membrane and thereby produces loss of fluidity and breakdown of the membrane secretory functions and transmembrane ionic gradient. The end products of lipid peroxidation are toxic, mutagenic and carcinogenic. Lipid peroxidation is strongly associated with various diseases including inflammation, hepatotoxicity (Gulec et al., 2006), mutagenicity and carcinogenicity (Sengottuvelan and Nalini, 2006).

LPO is a radical mediated chain process involving three sequences: initiation, propagation and termination and is presented in Figure 3. Most commonly, the reactive oxygen species superoxide, singlet oxygen, triplet oxygen, hydroxyl radical, alkoxy radical (RO) and peroxy radical activate a lipid molecule and initiate lipid peroxidation of lipid membranes. The free radical (R) attacks fatty acid chain of membrane lipids (LH) by abstracting a hydrogen atom from one of the carbon atoms in the side chains and combine with it to form water thus leaving a carbon – centered radical (-C-) in the membrane (reaction 1); this process is known as initiation stage.
FIGURE 3
MECHANISM OF LIPID PEROXIDATION

(Valko et al., 2006)
Hydrogen atoms that are abstracted from a methylene group (-CH2-) have very high mobility. This attack easily generates free radicals from polyunsaturated fatty acids (PUFA). The presence of a double bond in the fatty acid weakens the C-H bond on the carbon atom adjacent to the double bond and so makes hydrogen removal easier.

\[-\text{CH}_2^- \text{OH}^- \rightarrow \text{CH} + \text{H}_2\text{O}\]

Carbon centered radicals usually undergo molecular rearrangement to produce conjugated diene. Conjugated diene combines with molecular oxygen which leads to the formation of lipid peroxy radical (LOO·) which in turn is reactive enough to attack adjacent fatty acid side chains, abstracting hydrogen to from lipid hydroperoxide (LOOH). This is the propagation stage of LPO. One hydroxyl radical can result in the conversion of many hundred fatty acid side chains into lipid hydroperoxides.

The hydroperoxides react with transition metal ions (Fe^{2+} / Cu^{2+}) to form alkoxyl (LO·) and peroxyl radicals (LOO·) respectively. Lipid hydroperoxides can also decompose to yield a range of cytotoxic products the most unpleasant of which are aldehydes, malondialdehyde (MDA) and 4 – hydroxyl nonenal. L· can react with a lipid peroxy radical (LOO·) to give non-initiating and non – propagating species such as the relatively stable dimers (LOOL). The chain reaction continues until the PUFA substrate is completely consumed. This is known as the termination stage of lipid peroxidation (Valko et al., 2006)

2.7 ANTIOXIDANT DEFENSE IN COMBATING OXIDATIVE STRESS

Aerobic organisms possess sophisticated defense system for converting reactive oxygen species into less harmful or harmless compounds formed within the cells, i.e., the antioxidants (Eraslan et al., 2004a).

An antioxidant is any substance that can delay or prevent cellular oxidative damage caused by free radicals and may act in numerous ways,
ranging from prevention of their formation to their interception once formed (Weyers et al., 2008). In absorbing free radicals, antioxidants ‘trap’ (de-energize or stabilize) the lone free radical electron and make it stable enough to be transported to an enzyme, which combines two stabilized free radicals together to neutralize. The antioxidants, besides scavenging free radicals (Patel and Sail, 2006), induces drug metabolizing enzymes (Rose et al., 2005) and inhibit carcinogen induced mutagenesis and tumorigenesis at several target organ sites including aflatoxin B1 induced liver tumors (Lambert and Yang, 2003). Antioxidants can be broadly classified into enzymic [superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx)] and nonenzymic (vitamin C, vitamin E and GSH) antioxidants (Jankov et al., 2001).

2.8 STRATEGIES TO CONTROL AND DETOXIFY AFLATOXINS

The strategies to reduce the impact of aflatoxins include plant breeding for mold resistance, efficient harvesting and storage practices to prevent contamination and development of commercially applicable techniques for decontamination.

2.8.1 GOOD AGRICULTURAL AND MANUFACTURING PRACTICES

To prevent aflatoxin contamination, the preharvest strategies like maintenance of proper planting / growing conditions, antifungal treatments and adequate insect and weed prevention are recommended. During harvest, use of functional harvesting equipment, clean and dry collection / transportation equipment and appropriate harvesting conditions are also recommended. Further, the post harvest strategies like drying as dictated by the moisture content of the harvested grain, appropriate storage conditions, and use of transport vehicle that are dry and free of visible fungal growth have also been recommended to reduce aflatoxin contamination (Park, 2002; Strasnider et al., 2006).
2.8.2 DECONTAMINATION METHODS

Many decontamination methods have been tried to destroy already formed toxin from food or feed and reduce the toxic effects of the contaminated products. The decontamination methods include physical, chemical and biological methods.

Physical methods

Screening and sorting of aflatoxin contaminated seeds were identified as the most effective approaches in the case of peanuts when the seeds were blushed. However, Williams et al. (2004) reported that sorted nuts were fed to animals or consumed by the poorest producers and labourers. Yazdanpanah et al. (2005) reported a time and temperature dependent degradation of aflatoxin contaminated samples by roasting the samples. In case of rice, pressure cooking (Park and Kim, 2006) and parboiling (Toteja et al., 2006) were reported to remove AFB₁.

Addition of adsorbents bentonite, hydrated sodium calcium aluminosilicate (HSCAS) to contaminated feeds had proved to be effective in reducing the bioavailability of aflatoxins in animals (Oguz and Kurtoglu, 2000; Eraslan et al., 2004). Calcium montmorillonite, the adsorbent was demonstrated to be safe for humans (Wang et al., 2005), which would allow the use of this technique for products intended for human consumption. Microorganisms like yeasts and bacteria have been tested on their ability to modify or inactivate aflatoxins (Zaghini et al., 2005).

Chemical methods

Chemically, aflatoxins can be destroyed with calcium hydroxide, monomethyl amine, ammonia and ozone. Among all chemicals, ammoniation was extensively used for cotton seed meal, peanut meal or sunflower meals. But
the main drawback using chemicals was found to be their carryover to animals that resulted in the deterioration of animal health (Galvano et al., 2001).

**Biological methods**

*Flavobacterium aurantiacum* was shown to remove AFB₁ from liquid media and was used in peanut processing as biodegrader (Diarra et al., 2005).

### 2.8.3 CHEMOPREVENTION TO COUNTERACT AFLATOXIN TOXICITY

Compounds that either decrease activation or increase detoxification of aflatoxin were used in chemoprevention. Long term drug therapy seemed to be expensive and therefore difficult to implement in poor communities and had side effects (Williams et al., 2004). Use of plants as a source of medicine has been inherited and is an important healthcare system in India. India is the largest producer of medicinal herbs. Nutritive and non-nutritive compounds in a number of foods of plant origin have been shown to modulate AFB₁ biotransformation, binding to DNA and carcinogenesis. The plant and plant products that provide protection against AFB₁ are summarized in Figure 4.

**FIGURE 4**

PLANT AND PLANT PRODUCTS THAT PROVIDE PROTECTION AGAINST AFB₁

- Pepper, grapes, turmeric, garlic, Fava tonka (Galvano et al., 2001)
- Cabbage and onions (Wahhab and Aly, 2003)
- Broccoli (Kensler et al., 2005)
- Coffee and black tea (Choudhary and Verma, 2005)
- Sunflower oil and soybean oil (Raju et al., 2005)
- Laurencia obstusa, Caulerpa prolifera (Wahhab et al., 2006)
- Silybum marianum (Tedesco et al., 2004)
- Manodora myristica (Flora and Victor, 2004)
- Scutellaria baicalensis (Boer et al., 2005)
- Kolaviron from *Digitalis purpureae* (Lee et al., 2006)
- Lupeol (Preetha et al., 2006)
- Aquilegia vulgaris (Liebert et al., 2006)
2.9 *Moringa oleifera*

*Moringa oleifera* Lam (Synonym: *Moringa pterygosperma* Gaertner) belongs to a monogenic family, *Moringaceae* that is considered to have its origin in Agra and Oudh in the northwest region of India, South of Himalayan mountains. Although the name “shigon” for *Moringa oleifera* is mentioned in the “Shrushruta Sanhita”, written in the beginning of first century A.D., there is evidence that the cultivation of this tree in India dates back many thousands of years. This rapidly growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, monger, moonga, mulangay, nebeday, saijhan, sajna or ben oil tree) was utilized by Romans, Greeks and Egyptians also (Fahey, 2005; Kumar *et al.*, 2007).

2.9.1 MORPHOLOGY OF *Moringa oleifera*

*Moringa* is a fast growing, perennial tree that grows on hillsides, pasturelands and river basins. It can reach a maximum height of 7-12 m and a diameter of 20-40 cm at chest height. The fruits are three lobed pods and are 20 - 60 cm in length. Each pod contains between 12 and 35 seeds. Each tree can produce between 15,000 and 25,000 seeds/year and the average weight per seed is 0.3 g (Resmi *et al.*, 2005).

2.9.2 BIOLOGICAL IMPORTANCE OF *Moringa oleifera*

All parts of Moringa tree are edible. It is a rich source of carotenoids, calcium, iron and minerals. The seed kernel contains on an average 40 percent by weight of oil, with palmitic, stearic, behenic and oleic acids. *Moringa* species are rich in fairly unique group of phytochemicals, glucosinolates and isothiocyanates as reported by Bennett *et al.* (2003) and Fahey (2005). The *Moringa oleifera* leaves, drumsticks and their medicinal properties are given in Figures 5 and 6 respectively.
**FIGURE 5**

*Moringa oleifera* LEAVES AND THEIR MEDICINAL PROPERTIES

- Regulation of hyperthyroidism (Tahiliani and Kar, 2000)
- Radio-protective effect (Rao *et al.*, 2001)
- Vitamin A supplement (Nambiar and Seshadri, 2001)
- Antihypertensive effect (Dangi *et al.*, 2002)
- Hypoglycemic effect (Kar *et al.*, 2003)
- Antioxidant (Sidduraju and Becker, 2003)
- Enhanced milk yield (Sanchez *et al.*, 2005)

**FIGURE 6**

DRUMSTICKS AND THEIR MEDICINAL PROPERTIES

- Hepatoprotective effect (Pari and Kumar, 2002)
- Antioxidant effect (Ashokkumar and Pari, 2003)
- Anticancer effect (Bharali *et al.*, 2003)
The leafy vegetables are consumed by people and are well accepted by them. *Moringa* leaves at levels of 600mg/g diet did not produce any toxicity in mice as observed by Aruna and Sivaramakrishnan (1990).

2.10 *Aloe vera*

*Aloe vera barbadensis* Mill - otherwise known as *Aloe vera* belongs to the family, *Liliaceae* and is a popular medicinal plant found in many geographical regions. It is believed to be effective against a wide range of diseases and ailments in both humans and livestock (Das and Chattopadhay, 2004; Mwale *et al.*, 2005). Use of *Aloe vera* is mentioned in records as long as 400 BC and is thought to have originated in Egypt or the Middle East. The Egyptians referred to this plant as “The Plant of Immortality” (Hayes, 1999).

2.10.1 MORPHOLOGY OF *Aloe vera*

The plant has yellow flowers and triangular fleshy leaves with serrated edges that arise from a central base and may grow to nearly 2 feet long. The inner portion of the *aloe* leaves produces *aloe vera* gel (or mucilage) a clear, thin, tasteless jelly like material. The middle leaf layer produces bitter yellow exudates with powerful laxative-like actions. The fibrous outer part of the leaf serves a protective function (Wynn, 2005).

2.10.2 BIOLOGICAL IMPORTANCE OF *Aloe vera*

The principal ingredients of *aloe vera* gel in addition to water are aloin, barbaloin, ethereal oil, cinnamic acid, emodin, esters of cinnamic acid, calcium, potassium, aminoacids, glucose, mannose, uronic acids, vitamins and minerals (Wynn, 2005). Acetylated mannose or acemannan, biologically active polysaccharide in *aloe vera*, given to rats at a dose of 50mg / kg intraperitoneally or at a dose of 4.0 mg/kg intravenously did not produce any toxicity (Tarro, 1993). Feeding of 1% freeze dried *aloe vera* filet or 0.02% *aloe*
_Aloe vera_ in drinking water throughout the life span of rats did not produce any harmful or deleterious effects (Ikeno _et al._, 2002). _A. vera_ and its medicinal properties are given in Figure 7.

**FIGURE 7**

*Aloe vera* AND ITS MEDICINAL PROPERTIES

- Free radical scavenging activity (*Yagi et al.*, 2002)
- Wound healer (*Wynn*, 2005)
- Cytoprotective effect (*Norikura et al.*, 2002)
- Antioxidant activity (*Hu et al.*, 2003)
- Hypcholesterolemic effect (*Lim et al.*, 2003)
- Moisturizing effect (*Wynn*, 2005)
- Improvement of bowel health (*Langmead et al.*, 2004)
- Antidiabetic and antimutagenic effect (*Yoo and Lee*, 2005)
- Increased bioavailability of Vitamin C and Vitamin E (*Vinson et al.*, 2005)
- Hepatoprotective effect (*Etim et al.*, 2006)
- Treatment of Coccidiosis, Newcastle disease (*Mwale et al.*, 2005)
2.11 PROTECTIVE ROLE OF SILYMARIN IN AFB1 TOXICITY

Silymarin, an antioxidant and hepatoprotectant was reported to provide protection against the negative effects of AFB1 in rats (Rastogi et al., 2000) and broilers (Tedesco et al., 2004). The bioactive extract from Silybum marianum (milk thistle), silymarin contains a mixture of flavolignans, silybin, silydianin, isosilybin and silychristin, quercetin, taxifolin and dehydrodiconiferyl alcohol (Choksi et al., 2000). An antitoxic effect was also suggested by a lower milk excretion of aflatoxin M1, an AFB1 metabolite, in dairy cows fed with silymarin (Tedesco et al., 2003). Preetha et al. (2006) have used silymarin as a standard hepatoprotectant against AFB1-induced peroxidative hepatic damage in rats. Further, Castleman, (1995) has suggested that silymarin might not interfere with the liver metabolism of drugs, and thereby with the action of medications (Castleman, 1995).