Summary and Conclusion
Mycotoxins are toxic secondary metabolites of certain fungi and cause illness or death when ingested by animals or human beings. One of the most toxic group of mycotoxins are the aflatoxins produced by Aspergillus fungi. Aflatoxins have been detected in a wide range of commodities destined for consumption by humans, birds and animals. They are considered as an unavoidable contaminant of foods by the United States Food and Drug administration. Among the naturally occurring aflatoxins, aflatoxin B1 (AFB1) is the most predominant, toxic and carcinogenic compound. It is hepatotoxic, hepatocarcinogenic, teratogenic and mutagenic to rats, poultry and humans and has been classified as class 1A human carcinogen by the International Agency for Research on Cancer. In poultry, the toxic effects of aflatoxin include liver damage, growth retardation, immunosuppression and mortality. Moreover, consumption of AFB1 contaminated feed by poultry results in the carryover of toxic metabolites of AFB1 into the poultry products meat and eggs, which in turn may exert immunosuppressive, embryotoxic and teratogenic effects. Aflatoxins cause economic losses in poultry even at low levels of exposure. Aflatoxins pose great problem in the tropics than in the temperate regions but no part of the world can be considered to be aflatoxin free zone due to the movement of various foods and feedstuffs from one part of the globe to the other.

Medicinal plants, often serve as hepatoprotective agents in the prevention of toxicity caused by certain drugs and environmental chemicals. Moringa oleifera and Aloe vera are medicinal plants used in traditional medicines. However, so far no scientific studies are available on M. oleifera and A. vera as ameliorating agents against aflatoxin toxicity.
Hence this research work "Protective effect of *Moringa oleifera* and *Aloe vera on Aflatoxin B1 induced toxicity" was taken up. The feeds form the major route of aflatoxin exposure. Analysis of feed ingredients and mixed feeds commonly used to prepare mixed feeds for the presence of AFB1 might give a clear data on the extent of exposure of poultry to aflatoxin and its toxic effects. Therefore, poultry feeds and feed ingredients were screened for AFB1 contamination.

Animal studies have played a fundamental role in toxicological research studies, which might provide a vast knowledge about the health risks that aflatoxin can pose to humans and poultry. Hence the research work was also aimed to evaluate the biochemical and histopathological changes due to AFB1 toxicity and the effectiveness of the selected plant products of *M. oleifera* and *A. vera* in overcoming aflatoxin toxicity in rats and broilers.

The study was conducted in three phases. In phase I, a total of 200 samples representing mixed feeds (commercial poultry feed) of five brands and various poultry feed ingredients (groundnut cake, maize, sunflower cake, sorghum, bajra, ragi, rice bran, soybean meal and wheat bran) were collected from the feed manufacturers, feed dealers, retailers and farmers in and around Coimbatore and screened for aflatoxin B1 content.

Of the 200 samples tested, 42 per cent were contaminated with AFB1. The highest percentage of AFB1 contamination was observed in maize (61%), followed by groundnut cake (GNC) and mixed feeds (55%). The percentage of AFB1 contamination in sunflower cake, sorghum, ragi, bajra and rice bran were found to be 31, 33, 17, 31 and 17 per cent respectively.

The concentration of AFB1 was found to be between 17-649 ppb in the samples analyzed. Of the 22 AFB1 contaminated samples of GNC, one had toxin less than 20 ppb, 8 samples exhibited toxin between 21 to 100 ppb,
8 samples had toxin in the range of 101-200 ppb and 5 samples showed above 200 ppb. In maize, 20 samples had AFB1 between 21-100 ppb and 3 samples had toxin levels between 100-200 ppb and 5 samples had toxin above 200 ppb. Out of 16 sunflower cake samples, one sample showed AFB1 below 20 ppb while 4 others had AFB1 in the range of 21-100 ppb. The sorghum and bajra samples exhibited AFB1 in the range of 39-86 ppb and 38-147 ppb respectively. Two out of twelve rice bran samples screened were found to contain 17 and 40 ppb AFB1. Among 12 ragi samples, two samples showed AFB1 at the levels of 19 and 47 ppb. Out of 31 mixed feed samples, 17 exhibited AFB1 concentrations in the wide range of 18-212 ppb. Moreover, the same brand of feeds collected at different locations exhibited different levels of AFB1.

In phase II, the adverse effect of aflatoxin B1 and the protective effect of the selected plant products of *M. oleifera* and *A. vera* against AFB1 toxicity were tested in rats. Male Wistar rats weighing 50-55g and 21-25 days of age were used for the study. The rats were fed with basal diet and clean water *ad libitum*. The animals were maintained as per the principles and guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. The study was approved by the ethical committee (Vide No: 01, 2006), Avinashilingam University for Women, Coimbatore.

Ninety rats were divided into fifteen groups and were fed with basal diet or basal diet and plant products with or without aflatoxin B1 (AFB1). Aflatoxin B1 was administered at the dose of 50μg/kg BW. The plant products selected for the study were *Moringa oleifera* leaf juice (MJ), drumstick pulp powder (DP) and *Aloe vera* gel (AV). MJ at the doses of 0.5ml/kg BW (MJ1) and 1.0 ml/kg BW (MJ2) and AV at the doses of 0.5ml/kg BW (AV1) and 1.0 ml/kg BW (AV2) were fed orally. DP was fed with the diet at the dose of 2g/kg BW (DP1) and 3g/kg BW (DP2).
Among the fifteen groups, group 1 was fed with basal diet and group 2 was given AFB1. Groups 3, 4, 5 and 6 were fed with MJ1, AFB1+MJ1, MJ2 and AFB1+MJ2 respectively. Groups 7, 8, 9, 10 were fed with DP1, AFB1+DP1, DP2, AFB1+DP2 respectively. Groups 11, 12, 13 and 14 were fed with AV1, AFB1+AV1, AV2 and AFB1+AV2. Group 15 was fed with AFB1 along with the standard drug silymarin (10mg/kg BW).

The experiment was conducted for a period of 30 consecutive days after which the rats were sacrificed. The toxicity induced by aflatoxin was proved in terms of the altered hematological, biochemical parameters and histopathological findings. Feeding aflatoxin to rats decreased hemoglobin, red blood cell count and packed cell volume and increased white blood cell count, compared to the control group. The serum proteins and cholesterol were decreased and serum uric acid and blood glucose were increased. Supplementation of the plant products M. oleifera leaf juice, drumstick pulp power and A. vera gel along with AFB1 reversed the changes caused by AFB1 on hematological and serum biochemical parameters.

Aflatoxin toxicity was found to elevate the hepatic marker enzymes AST, ALT, ALP and GGT in serum and the increase was overcome by supplementation of the plant products M. oleifera leaf juice, drumstick pulp power and A. vera gel along with AFB1.

The lipid peroxidation products, TBARS, hydroperoxides and conjugated dienes in liver and kidney were increased whereas the enzymic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and the nonenzymic antioxidants (vitamin C, vitamin E and reduced glutathione) were decreased in plasma, liver and kidney of rats fed with AFB1. The increase in lipid peroxidation products and the decrease in antioxidants were overcome by supplementation of the plant products M. oleifera leaf juice, drumstick pulp power and A. vera gel.
Aflatoxin feeding resulted in severe congestion, vacuolation, blood in sinusoids and degeneration in liver and complete loss of tubular structure, congestion, loss of nuclear details and vacuolation in kidney of rats. Supplementation of the plant products *M. oleifera* leaf juice, drumstick pulp powder and *A. vera* gel along with AFB1 did not reveal any pathological changes in liver and kidney.

The results revealed that, of the two concentrations of the plant products used in, the higher dose of *Moringa oleifera* leaf juice, drumstick pulp powder and *Aloe vera* gel was highly effective in reversing the toxic effects of AFB1.

In phase III, the adverse effect of aflatoxin B1 and the protective effect of the selected plant products of *M. oleifera* and *A. vera* against AFB1 toxicity were tested in broilers.

One hundred and seventy six commercial, straight run day-old broiler chicks belonging to a single hatch were purchased from a commercial hatchery at Coimbatore. A shed in a private farm was rented out near Thudiyalur in Coimbatore district, TamilNadu and the experiment was carried out. All broilers were fed *ad libitum* with a basal diet, fed in two stages: starter diets (0-3 weeks) and finisher diets (4-6 weeks). The study was approved by the ethical committee (Vide No: 01, 2006), Avinashilingam University for Women, Coimbatore.

The plant products of *Moringa oleifera* and *Aloe vera* and the doses of the plant products were selected based on their applicability in the field conditions and leaf powder of *Moringa oleifera* (ML), pulp powder of drumsticks (DP) and the gel of *Aloe vera* (AV). ML, DP and AV were fed at the dose of 3.0g/kg of feed. Besides feeding individually, ML, DP and AV were designated as herbal product (HP) and fed together, each at the dose of 1g/kg feed. Aflatoxin B1 was fed at the dose of 100 μg/kg feed (100 ppb).
The birds were divided into eleven groups of 16 birds each. Group 1 was fed with basal diet and group 2 was given AFB1. Groups 3, 5, 7 and 9 were fed with ML, DP, AV and HP respectively. Groups 4, 6, 8 and 10 were fed with AFB1 + ML, AFB1 + DP, AFB1 + AV and AFB1 + HP respectively. Group 11 was fed with AFB1 along with the standard drug silymarin at the dose of 100 mg/kg feed (100ppm).

The experiment was conducted for a period of 42 consecutive days after which the broilers were sacrificed. The body weight, hematological, biochemical parameters and hepatic marker enzymes in serum, lipid peroxidation and antioxidant status and histopathological changes in liver tissue of broilers were studied.

The body weight and body weight gain of broilers were not affected by aflatoxin feeding till the end of second week. From the third to the sixth week, aflatoxin feeding significantly decreased the body weight and body weight gain of broilers when compared to that of controls. The body weight and body weight gain were improved by supplementation of *Moringa oleifera* leaf powder, drumstick pulp powder and *Aloe vera* gel in the group fed with aflatoxin. However, at the end of the experimental period, it was observed that the broilers body weight and body weight gain were on par with controls, only in groups supplemented with herbal product along with AFB1. Though the body weights were decreased, the percent cut up parts yield, the organ weights and hematological parameters in broilers were not affected in groups fed with 100 ppb aflatoxin.

The results revealed decreased levels of serum proteins and cholesterol and increased levels of blood glucose in broilers treated with AFB1. The toxic effects of aflatoxin on serum biochemical parameters were reversed by the supplementation of the plant products *M. oleifera*, and *A. vera* in groups fed with AFB1. Supplementation of the plant products of *M. oleifera* and *A. vera*
was effective in overcoming the increase in hepatic marker enzymes manifested in aflatoxin toxicity

Aflatoxin feeding in broilers increased the lipid peroxidation products in liver with a concomitant decrease in enzymic and nonenzymic antioxidants. The alterations in lipid peroxidation products and the antioxidants were reversed by supplementation of the products of *M. oleifera* and *A. vera*.

Results of histopathology revealed multifocal necrotic areas, microvesicular fatty changes in hepatocytes, acinar transformation, severe sinusoidal congestion and disrupted hepatic cords. The pathological changes were reduced by supplementing the products of *M. oleifera* and *A. vera*.

The results of phase III clearly indicated the protective effect of the plant products *M. oleifera* and *A. vera* against the toxicity induced by aflatoxin. Further, the results also demonstrated that the plant products were highly effective when supplemented together, rather individually.

The following conclusions may be drawn from the present investigation:

1. The feed ingredients, groundnut cake and maize were observed to be the major sources of aflatoxin contamination.

2. The hematological, biochemical and histopathological changes which occurred by feeding aflatoxin in rats and broilers could be overcome by supplementation of the products of *Moringa oleifera* and *Aloe vera*.

3. In rats, among the selected doses of plant products, the higher dose of the plant products [MJ (1.0 ml/kg BW), DP (2.0 g/kg BW) and AV (1.0 ml/kg BW)] was effective in overcoming the toxicity induced by aflatoxin. In broilers, the plant products were highly effective against aflatoxin when supplemented together [HP: ML (1.0 g/kg feed), DP (1.0 g/kg feed) and AV (1.0 g/kg feed)] instead of supplementing individually.
Scope for further studies

- Routine survey of poultry feed ingredients and poultry feeds for contamination of aflatoxin to give the extent of exposure of the poultry to aflatoxin to help in taking measures against aflatoxin induced toxicity

- Prevention of the growth of aflatoxin producing organisms by following stringent measures including improvement of feed storage practices

- Assessment of the plant products of *Moringa oleifera* leaf juice, drumstick pulp powder and *Aloe vera* gel against AFB1–induced hepatocarcinogenesis

- Isolation and characterization of the active principles in *Moringa oleifera* and *Aloe vera*, which play the protective role against aflatoxin

- Quantification of the effect of *Moringa oleifera* and *Aloe vera* against high levels of aflatoxin i.e., above 1.0 ppm in poultry

- Incorporation of *Moringa oleifera* leaves and drumstick pulp in different forms into the feeds and mixing *Aloe vera* gel with water given to poultry to prevent aflatoxin toxicity which can prevent the entry of contaminated meat to the consumers through the food chain