CHAPTER V

SUMMARY AND CONCLUSIONS
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The present study was undertaken to evaluate the effects of oral administration of aflatoxin on vital organs like liver and kidney along with reproductive organs such as testis, caput and cauda epididymides, seminal vesicle and ventral prostate gland in male mice. Furthermore, the possible amelioration of aflatoxin-induced effects on treatment with curcumin was also evaluated.

Aflatoxin treatment (750 and 1,500 μg/kg body weight/day) for 45 days caused dullness and lethargy with signs of staggering. Furthermore, autopsy in aflatoxin-treated animals revealed fragile liver. Gas filled gastrointestinal tract and pale coloured liver and kidney were also observed in aflatoxin-treated mice. No treatment related clinical signs were observed in controls and aflatoxin plus curcumin-treated animals.

BODY WEIGHT

Oral administration of aflatoxin caused dose-dependent significant reduction in body weight of mice, as compared to controls. Administration of curcumin along with aflatoxin caused significant amelioration in aflatoxin-induced reduction in body weight.

LIVER

Aflatoxin treatment for 45 days caused, as compared with controls, significant and dose-dependent increase in absolute and relative liver weights in mice. Administration of aflatoxin along with curcumin significantly ameliorates aflatoxin-
induced changes. Extent of amelioration was more in high dose aflatoxin plus curcumin- 
treated mice than that of low dose.

No significant difference in DNA, RNA and protein contents were observed in the 
liver of untreated, vehicle and curcumin-treated controls. Oral administration of aflatoxin 
caused, as compared with controls, significant, dose-dependent reduction in DNA, RNA 
and protein content. Aflatoxin-induced changes were significantly ameliorated on 
aflatoxin plus curcumin treatment in the liver of mice. Amelioration was almost complete 
in all parameters in low dose group (Group 6).

There was no significant change in the activities of succinate dehydrogenase and 
adenosine triphosphatase between untreated, vehicle control and curcumin alone treated 
groups. However, oral administration of aflatoxin caused dose-dependent, significant 
reductions in the activities of succinate dehydrogenase and adenosine triphosphatase, as 
compared to the controls. Treatment with curcumin along with aflatoxin caused 
significant amelioration in all parameters as compared to aflatoxin alone treated groups. 
Almost complete amelioration was observed in activities of succinate dehydrogenase and 
adenosine triphosphatase in low dose aflatoxin plus curcumin-treated mice, while it was 
partial in high dose aflatoxin plus curcumin-treated group.

There were no significant alterations in lipid peroxidation, and glutathione and 
total ascorbic contents, as well as activities of catalase, superoxide dismutase and 
glutathione peroxidase between untreated, vehicle control and curcumin alone treated 
groups. Aflatoxin treatment caused dose-dependent, significant rise in the level of lipid 
peroxidation by reducing the non-enzymatic antioxidants such as glutathione and total 
ascorbic acid as well as enzymatic antioxidants such as superoxide dismutase, glutathione
peroxidase and catalase as compared to controls in the liver of mice. Aflatoxin-induced changes were significantly ameliorated on curcumin treatment. Amelioration was almost complete in low dose aflatoxin-treated group. However, partial amelioration was observed in glutathione levels and in the activity of catalase in high dose group, as values were still significantly different in comparison to the controls.

The present study clearly revealed zonal necrosis, nuclear pyknosis, cytoplasmic vacuolization along with fatty infiltration in the liver of aflatoxin treated mice. Decrease in hepatocellular compactness was also observed. Effect was significantly higher in high dose group than that of low dose. No such significant changes were observed in all groups of controls as well as curcumin plus aflatoxin-treated groups of animals.

**KIDNEY**

There was no significant change in absolute and relative kidney weights between different control groups. However, aflatoxin treatment for 45 days caused significant, dose-dependent increase in absolute and relative kidney weights as compared to controls. Oral administration of curcumin along with aflatoxin cause significant amelioration in absolute and relative kidney weights in mice.

There was no significant change in DNA, RNA and protein contents as well as activities of succinate dehydrogenase and adenosine triphosphatase among untreated, vehicle control and curcumin alone treated groups. Oral administration of aflatoxin (750 and 1,500 μg/kg body weight/ammal/day) for 45 days caused a significant reduction in DNA, RNA and protein contents, as well as activities of succinate dehydrogenase and adenosine triphosphatase as compared to controls. The effect was dose-dependent. Oral
administration of curcumin along with aflatoxin caused significant amelioration in DNA, RNA and protein contents as well as activities of succinate dehydrogenase and adenosine triphosphatase as compared to aflatoxin alone treated group.

There was no significant difference in enzymatic and non-enzymatic antioxidants in the kidney of untreated control, vehicle control and curcumin alone treated groups. Lipid peroxidation was significantly increased in the kidney of mice treated with aflatoxin which could be due to lower levels of non-enzymatic antioxidant like glutathione and total ascorbic acid as well as the enzymatic antioxidants like catalase, superoxide dismutase and glutathione peroxidase activities. All treatment related changes were significantly ameliorated on aflatoxin plus curcumin treatment. However, there was partial amelioration in the levels of superoxide dismutase and glutathione in the high dose group as values were significantly different than the controls.

No apparent histopathological changes were observed in the kidney of all groups of controls. Normal Bowman’s capsule with glomerulus and proximal and distal convoluted tubules were observed. Aflatoxin treatment for 45 days caused distortion of the tubules, increased vacuolization, necrosis, disorganisation of the glomerulus and increased space between glomerulus and capsule wall. Oral administration of curcumin along with aflatoxin caused amelioration in all aflatoxin-induced changes in kidney of mice.

REPRODUCTIVE ORGANS

The present study was carried out to evaluate the effects of oral administration of aflatoxin on male reproductive functions. Furthermore, the possible amelioration of aflatoxin-induced effects on curcumin treatment was also evaluated.
Oral administration of aflatoxin caused dose-dependent, significant reductions in absolute and relative weights of testis, caput and cauda epididymides, seminal vesicle and ventral prostate gland. Administration of curcumin along with aflatoxin significantly ameliorates aflatoxin-induced changes.

Oral administration of aflatoxin for 45 days caused dose-dependent, significant reductions in protein, DNA, RNA and cholesterol contents in the testis of mice as compared with the control. As compared with the control, the activities of \(3\beta\)- and \(17\beta\)-hydroxysteroid dehydrogenases in the testis were significantly lower in aflatoxin-treated mice. Aflatoxin-induced changes were significantly ameliorated on curcumin treatment.

Aflatoxin treatment for 45 days caused significant reductions in the activities of ATPase, SDH and sialic acid contents in the testis of mice. However, significant rise in acid phosphatase activity was noted in the ventral prostate gland of aflatoxin-treated mice. Treatment with curcumin caused significant recovery in all parameters.

Lipid peroxidation was significantly higher in the testis of aflatoxin-treated mice which could be due to lower levels of non-enzymatic antioxidants (glutathione, total ascorbic acid) as well as the activities of enzymatic antioxidants (superoxide dismutase, glutathione peroxidase and catalase). Curcumin treatment significantly ameliorated aflatoxin-induced changes.

Oral administration of aflatoxin for 45 days caused degenerative changes in the testis with evidence of distortion and confluence in all the seminiferous tubules, intraepithelial vacuolization, along with depletion of germ cells. Leydig cell degeneration and lumen filled with cellular debris were also observed in testis of aflatoxin-treated
mice. However, all treatment related changes were ameliorated on treatment with curcumin.

Aflatoxin treatment caused, as compared with the control, significant, dose-dependent reduction in protein and sialic acid contents in caput epididymis. Activities of ATPase and SDH were significantly reduced in caput epididymis of aflatoxin-treated mice. Curcumin treatment significantly ameliorated aflatoxin-induced changes.

Aflatoxin treatment for 45 days caused pyknosis of epithelial cell nuclei, clumping of stereocilia and lumen devoid of sperm bundles in caput epididymis. Treatment with curcumin for 45 days along with aflatoxin showed significant recovery as compared to aflatoxin alone treated groups.

Oral administration of aflatoxin caused dose-dependent, significant reduction in protein and sialic acid contents as well as activities of SDH and ATPase in cauda epididymis. Aflatoxin-induced changes were significantly ameliorated on treatment with curcumin as compared to the mice treated with aflatoxin alone.

Aflatoxin treatment caused degeneration with pyknosis of epithelial cell nuclei, disorganization of the epithelium and clumping of stereocilia in the tubules of cauda epididymis. The lumen was devoid of sperm bundles. However, recovery in aflatoxin-induced changes was observed on curcumin treatment.

As compared with control, aflatoxin treatment for 45 days caused a significant dose-dependent decrease in fructose content in seminal vesicle of mice. Treatment with curcumin along with aflatoxin brought about a significant recovery in the fructose content as compared with the aflatoxin alone treated groups of mice.
The protein content was significantly decreased in the ventral prostate gland of aflatoxin-treated groups of mice. On the other hand, the acid phosphatase activity was significantly increased, as compared to the control, in aflatoxin-treated group of mice. Curcumin treatment along with aflatoxin brought about significant recovery as compared to aflatoxin alone treated groups of mice.

Aflatoxin treatment for 45 days caused distortion of the tubules, and degeneration of smooth muscles in seminal vesicle and ventral prostate gland. Treatment of curcumin along with aflatoxin showed recovery in aflatoxin-induced changes.

SERUM

No significant changes in creatinine and protein contents, SGOT and SGPT activities were observed in the serum of different control groups of mice. Aflatoxin treatment caused significant increase in the level of creatinine as well as SGOT and SGPT activities. The effect was dose-dependent. However, protein content was significantly decreased in the serum of aflatoxin-treated mice as compared to controls. Curcumin treatment along with aflatoxin significantly ameliorated aflatoxin-induced changes in the serum of mice.

BLOOD

No significant change in RBC count and haemoglobin content were noted in the blood of different control groups of mice. Both RBC count and haemoglobin content, however, decreased significantly in aflatoxin-treated mice. The effect was dose-
dependent. Curcumin treatment along with aflatoxin significantly ameliorated aflatoxin-induced changes in blood parameters of mice.

**SPERM PARAMETERS**

As compared with the controls, aflatoxin treatment caused dose-dependent, significant reduction in cauda epididymal sperm count, viability, motility and fertility rate. Increase in sperm morphological abnormalities were also observed in aflatoxin-treated mice. Treatment with curcumin showed recovery in aflatoxin-induced changes in sperm parameters.

**IN VITRO STUDIES**

Addition of aflatoxin (0.5 - 2.0 µg/ml) to RBC suspension caused significant increase in percent haemolysis. The effect was concentration-dependent. The concurrent addition of curcumin/turmeric extracts (ethanolic and aqueous) (1 - 100 µg/ml) along with aflatoxin (2.0 µg/ml) caused significant retardation, which was concentration-dependent. Curcumin was found to be most effective than that of ethanolic and aqueous extracts of turmeric respectively.

Addition of aflatoxin (2 - 10 µg/ml) to human semen suspension caused significant decrease in viability, motility and increases morphological abnormalities in sperm. The effect was concentration- and time-dependent. Concurrent addition of curcumin/turmeric extracts (ethanolic and aqueous) (2 - 10 mg/ml) along with aflatoxin (10 µg/ml) caused significant recovery in viability, motility and reduction in
morphological abnormalities in sperm. This recovery was concentration- and time-
dependent.

*In vitro* studies on liver, kidney and testis homogenates revealed that \( \text{H}_2\text{O}_2 \)-
induced lipid peroxidation increased on addition of aflatoxin. The effect was dose-
dependent. Maximum increase was observed at 6 \( \mu\text{g/ml} \) concentration of aflatoxin.
Concurrent addition of curcumin (25 - 200 \( \mu\text{g/ml} \)) along with 6 \( \mu\text{g/ml} \) aflatoxin caused
significant reduction in lipid peroxidation in liver, kidney and testis homogenates which
was concentration-dependent. Curcumin was found to be more effective than other
extracts of turmeric (ethanolic and aqueous).

The present study clearly indicates that consumption of aflatoxin-contaminated
diet cause adverse effects in vital organs like liver and kidney as well as in reproductive
organs in mice. The safe tolerance limit of aflatoxin in most of the countries is 30 ppb.
However, regular occurrence of aflatoxin in the food/feedstuffs have been recognized in
most tropical and subtropical countries due to congenial environmental conditions for
moldy growth and toxin production.

Biological risk of exposure to aflatoxin is much lesser in technologically
developed countries than in developing ones. Also, reduced exposure to aflatoxin is
technically and economically feasible through the prevention of contamination of food
products or raw materials by rapid post-harvest drying of crops and controlled storage
conditions. Contamination of finished foods/feeds with molds invariably leads to the
exclusion of that product from the food chain in developed economies.

However, in most developing countries this is generally not possible and moldy
food such as cereals are often a regular part of the daily diet. Measures can be taken to
curb its impeding effects in human beings and animals by adopting suitable preventive measures such as healthy living with nutritious diet.

Oral administration of curcumin along with aflatoxin significantly ameliorates most of aflatoxin-induced effects in mice. It is one of the commonly consumed constituent of spice (turmeric) in Asian countries. Although curcumin treatment for long term causes some toxicity in reproductive functions, but on the other hand it has many beneficial effects as observed in the present study. Thus it follows Paracelsus medical concept: "All substances are poisons, there is none which is not a poison, only the right dose differentiates a poison and a remedy". So care should be taken in its consumption.

Although the experiments reported in this study could reveal many interesting facts, more challenging work could still be carried out but for the sophisticated analytical facilities. Similarly, selection of animal model, methods for analysis, etc. have been chosen taking into consideration availability of funds and facilities.

**Future Plan of Work**

- Extensive survey should be carried out on human beings starting from aflatoxin contamination of food/feed-stuffs.
- Exposure of aflatoxin in human beings can be measured by analysing aflatoxin-albumin adduct in serum.
- Also epidemiological studies should be done on aflatoxin exposure and various aflatoxin related problems in human beings.
- A correlation between consumption of curcumin/turmeric and aflatoxin related changes could also be established.
> Turmeric/curcumin has been used in ayurvedic medicine since ancient times, with various biological applications. Although some work has been done on the possible medicinal application, but no studies for drug-development have been carried out as yet. Thus, for its clinical applications extensive research on its bioactivity, mechanism of action, pharmacotherapeutics and toxicity studies should be carried out.

> Curcumin being a non-toxic, highly promising natural "eco-friendly" antioxidant compound having a wide spectrum of biological functions, should be studied deeply.

> It is expected that curcumin may find application as a useful drug in the near future to control various diseases, including HIV, Alzheimer and Parkinson disease etc. along with inflammatory disorders, carcinogenesis and oxidative stress-induced pathogenesis and also active cosmetic and antiseptic agent.

By this it can be said that once again for the welfare of mankind we will have to return to the lap of nature to get boon from treasure of eco-friendly antioxidants present very close to us.