CHAPTER V
SUMMARY AND CONCLUSION

The present study was carried out to evaluate the effects of DEA on human spermatozoa in \textit{in vitro} and oral administration of DEA on male reproductive functions of mice \textit{in vivo}. Furthermore, the possible amelioration of DEA-induced effects on co treatment with curcumin was also evaluated.

Addition of DEA to human sperm suspension caused concentration- and time-dependent significant decrease in sperm motility, viability and concentration-dependent significant increase in different kinds of sperm morphological abnormalities \textit{in vitro}. Concurrent addition of curcumin along with DEA caused significant recovery in sperm motility and viability. This recovery was concentration- and time-dependent. Similarly curcumin also caused concentration-dependent recovery in sperm morphological abnormalities.

Oral administration of DEA for 45 days caused dullness and lethargy. No treatment related clinical signs were observed in DEA plus curcumin-treated animals.

Diethanolamine treatment for 45 days caused dose-dependent, significant reduction in body weight, as well as, absolute and relative weights of testis, caput and cauda epididymis, vas deferens, seminal vesicle and prostate gland as compared to control. Co treatment with curcumin along with DEA significantly ameliorates DEA-induced changes in organ weights as compared to DEA-HD alone treated mice.

Oral administration of DEA for 45 days caused dose-dependent, significant reduction in protein, DNA, RNA and sialic acid contents in the testis of mice as compared to untreated control. These DEA-induced changes were significantly ameliorated by curcumin treatment.
Oral administration of DEA for 45 days caused significant increase in activities of ACP and ALP in testis of mice as compared to untreated control. Diethanolamine also caused significant reduction in activities of ATPase and SDH in testis of mice. However, co treatment of curcumin along with DEA caused significant recovery in all enzymatic activities as compared to DEA-HD alone treated mice.

Diethanolamine treatment for 45 days caused significant decrease in total lipid and cholesterol contents as well as activities of 3β- and 17β-hydroxysteroid dehydrogenase in testis of mice as compared to untreated control. As compared to untreated control, DEA also caused reduction in serum testosterone level. However, all DEA treatment related changes were significantly ameliorated by curcumin as compared to DEA-HD alone treated mice.

Oral administration of DEA for 45 days caused, as compared to untreated control, significant, dose-dependent increase in lipid peroxidation in testis of mice which could be due to reduction in activities of enzymatic antioxidants such as CAT, SOD and GSH-Px as well as non-enzymatic antioxidants such as TAA and GSH. Co treatment with curcumin along with DEA-HD significantly ameliorated DEA-induced changes as compared to DEA-HD alone treated mice.

Diethanolamine treatment for 45 days caused cellular pyknosis, degeneration of seminiferous tubules, depletion of spermatogenic cells and lower sperm concentration in testis. Degeneration of Leydig cells were also observed in testis. However, all treatment related degenerative changes were ameliorated by curcumin treatment.

Oral administration of DEA for 45 days caused, as compared to untreated control, significant dose-dependent reduction in protein and sialic acid contents in caput and cauda epididymis. Diethanolamine also caused significant decrease in activities of ATPase and SDH while increased activity of ACP was recorded in caput and cauda epididymis of
mice. Curcumin plus DEA co treatment significantly ameliorated all DEA-induced changes in caput and cauda epididymis as compared to DEA-HD alone treated mice.

Oral administration of DEA for 45 days caused degeneration in epithelium with clumping of stereocilia and lumen devoid of sperm in caput epididymis. Co treatment of curcumin plus DEA for 45 days showed significant recovery in caput epididymis.

Oral administration of DEA for 45 days caused decrease in stereocilia, degeneration in epithelium, reduction in sperm density and wider space between tubules. However, co treatment of curcumin along with DEA-HD for 45 days showed significant recovery in cauda epididymis.

Oral administration of DEA caused significant, dose-dependent reduction in cauda epididymal sperm count, motility, viability and male fertility index as compared to untreated control. Diethanolamine treatment also significantly increased different kinds of sperm morphological abnormalities as compared to untreated control. Curcumin along with DEA brought about significant recovery in DEA-induced changes in all sperm parameters and fertility index as compared to DEA-HD alone treated mice.

As compared with the control, DEA-treatment caused significant, dose-dependent reduction in protein content and ATPase activity as well as significant increase in glycogen content in vas deferens of mice. Curcumin plus DEA-HD treatment significantly ameliorated DEA-induced changes as compared to DEA-HD alone treated mice.

Oral administration of DEA caused clumping of stereocilia and absence of sperm bundle in lumen. However, co administration of curcumin along with DEA caused recovery in DEA-induced changes in vas deferens.

A significant, dose-dependent reduction in fructose content was observed in seminal vesicle of DEA-treated animals as compared with untreated control. Curcumin
plus DEA treatment caused significant amelioration in seminal vesicle as compared to DEA-HD alone treated mice.

Administration of DEA for 45 days caused degeneration in mucosal epithelium and mucosa folds in seminal vesicle which was significantly ameliorated by co-administration of curcumin along with DEA.

The protein content was significantly decreased in prostate gland of DEA-treated mice. On the other hand, ACP activity was significantly increased in DEA-treated mice as compared to control. Co-treatment of curcumin along with DEA caused significant amelioration in prostate gland of mice as compared to DEA-HD alone treated mice.

Diethanolamine treatment for 45 days showed degenerated luminal folds and epithelial cells in prostate gland of mice. Curcumin along with DEA brought about recovery in prostate gland of mice.

Curcumin has desirable preventing or putative therapeutic properties and it is useful for treatment of various diseases. We have tested curcumin up to 50 mg/kg bw in mice. It can be extrapolated to human dose using body surface area which is 4.05 mg/kg for 60 kg human being (Reagan-Shaw et al., 2007). Curcumin comprises of 2 to 8% of most turmeric preparations. So the calculated dose of curcumin for human being is available in routine diet. It has been previously mentioned that curcumin did not show any toxicity in human when administered at doses of 1-8 gm/day. Therefore, we recommend the daily usage of curcumin form of turmeric to overcome adverse effect of DEA and other such toxicants to which human beings are routinely exposed.

FUTURE PERSPECTIVES

The present study was an attempt to evaluate the DEA-induced reproductive toxicity and its possible amelioration by curcumin. The present work provides various biochemical and histopathological evidence for DEA-induced toxicity and its amelioration by
curcumin. Results revealed interesting facts and findings. However, the study can be extended further in the following ways:

(1) Extensive survey should be carried out to quantify the concentration of DEA in different consumer products such as cosmetics, shampoos and hair conditioners.

(2) Exposure of DEA in human beings can be measured by analyzing DEA and its metabolites in blood and its impact on human reproduction.

(3) Molecular mechanism of DEA-induced toxicity on testis and other related organs can be studied.

(4) Detail study on correlation between DEA exposure on choline deficiency and free radical generation can be explored.

(5) Oral administration of curcumin reduces DEA-induced toxicity in testis and other reproductive organs. However, mechanism regarding this interaction is not clear and it should be explored.