Areca nut chewing is a very popular habit not only in India but also worldwide. This practice dates back several thousand years and is deeply entrenched with the culture of the population. In India there are 75,000-80,000 new cases of oral cancer each year and the incidence rates of cancers of the oral cavity in both males and females in all urban cancer registries are among the highest in the world. There is a close association between chewing habit of areca nut with or without tobacco and cause of oral cancer and a variety of oral mucosal diseases, such as leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma. In order to confirm this, researchers are focusing on the toxic effects of arecoline, the major alkaloid present in the areca nut using different test systems. However, during metabolism of arecoline various metabolites can be formed inside the body. Despite its social and toxicological importance, relatively little is known about the metabolism of arecoline. There may be possibility of formation of more toxic metabolites before body completely detoxifies the parent compound. Moreover, the role of metabolites in inducing cytotoxicity and genotoxicity is well established. No study has been undertaken to determine areca nut derived secondary metabolites.
Therefore, in the present study three major metabolites of arecoline, arecaidine, arecoline N-oxide and N-methyl nipecotic acid were taken into consideration. Keeping in mind the large number of people around the world consuming areca nut as well as its importance of in clinical use against Alzheimer's disease, it is very important to understand the toxicological, carcinogenic potentials involved with such secondary metabolites of the major areca nut alkaloids.

The International Agency for Research on Cancer (IARC) initiated a programme to evaluate carcinogenic risk of chemicals to which human gets exposed. There is an increasing effort worldwide to determine the impact of environmental, genetic and life-style factors on genomic stability in human populations.

From the available literature, it is revealed that arecoline induces abnormal sperm heads in mice by damaging the germ cell nuclear DNA and also unscheduled DNA synthesis in early spermatid stages of the mouse, displays genotoxic effects and inhibits the growth of oral mucosal fibroblasts, gingival fibroblasts and keratinocytes mitochondrial activity and intracellular thiols.

Overproduction of reactive oxygen species can cause cell damage by attacking essential biomolecules such as lipids, proteins, enzymes and nucleic acids. Reactive oxygen species play crucial roles in multistage carcinogenesis. Epidemiological studies in Papua New Guinea revealed a strong association between reactive oxygen species and the progression of oral cancer. Some
reports have also shown that areca nut extract induces production of a variety of cytokines and prostacyclin by keratinocytes. This inferred the involvement of keratinocyte inflammation in areca nut extract promoted pathogenesis of oral cancers. The enhancing effects of dietary administration of areca nut on carcinogenesis in the liver and upper digestive tract were also observed. However, all these data are insufficient, contradictory or not available especially with arecoline metabolites.

The general conclusion is that (i) there is sufficient evidence in experimental animals for carcinogenicity of areca nut (ii) there is limited evidence in experimental animals about the carcinogenicity of arecoline (iii) there is insufficient evidence in experimental animals for the carcinogenicity of arecaidine or other metabolites.

Therefore, the present research work is undertaken in an effort to understand and find out whether areca nut alkaloid arecoline and its metabolites arecaidine, arecoline N-oxide and N-methyl nipecotic acid have any genotoxic and tumorigenic potential.

Considering the above mentioned facts and in view of the importance of this study in absence of any report on primary metabolites of arecoline, betel quid with or without tobacco chewing habit and the impact of arecoline induced carcinogenesis in chewers, the basic objectives of the proposed study are as follows:

1. To evaluate the genotoxic potential of arecoline and metabolites \textit{in vivo} in murine test system.
2. To evaluate the tumorigenic potential of arecoline and its metabolites in vivo in murine test system.

3. To compare the above results with whole betel quid with or without tobacco induced genotoxicity and tumorigenicity.

4. Biochemical analysis like lipid peroxidation level as other toxicological parameter for evaluation of cellular dysfunction due to oxidative stress in arecoline, its metabolites and betel quid exposed animals.

The major findings and observations of the present research work are as follows:

(I). The areca nut alkaloid arecoline have a strong genotoxic potential in both somatic (bone marrow cells) and germ (sperms) cells of mice. In all the three cytogenetic assays (chromosomal aberration, micronucleus, sperm head abnormality) and total sperm count studied, it was found that arecoline (20, 50 and 80 mg/kg/bw) induced a dose dependent linear increased in the genotoxicity. The highest dose was showing a statistically highly significant increase at (P<0.001).

(II). The major metabolite of arecoline, arecaidine at three different doses (20, 50 and 80 mg/kg/bw) induced a dose dependent increased in the frequency of chromosomal aberration, micronucleus, sperm head abnormality and dose dependent decreased in total sperm count. Middle and highest dose induced genotoxicity to a highly significant level (p<0.05) and (P<0.001) respectively as compared to the control group. A time dependent decreased in genotoxicity was also observed in case of chromosomal aberration and micronucleus assays after 24 and 48 hour studies.
(III). Arecoline N-oxide is another metabolite of arecoline. A dose dependent linear increased in the frequency of chromosomal aberration and micronucleus and was observed after 24 hour and 48 hour treatment. It was observed that arecoline N-oxide (50 mg/kg bw) and (80 mg/kg bw) show increase in genotoxicity which was highly significant at (P<0.001). In case of sperm head abnormality, it was found that a dose dependent increased and dose dependent reduction in total sperm count after 35 days of exposure.

(IV). N-methyl nipecotic acid is also one of the major metabolite of arecoline. A dose dependent linear increased in the frequency of chromosomal aberration and micronucleus and was observed after 24 hour and 48 hour treatment. The highest dose (80 mg/kg bw) was showing a statistically significant (p<0.01) level of increase. The percentage of M1 remains almost similar in both 24 hour and 48 hour exposed groups within the tested dose of N-methyl nipecotic acid. A time dependent declined in genotoxicity was also observed. A dose dependent increase in induction of abnormal sperm after 35 days was observed. Also a dose dependent decreased in total sperm count was observed at (P<0.001) level of significance.

(V). Comparative study reveals that out of the four tested chemicals (arecoline, arecaidine, arecoline N-oxide and N-methyl nipecotic acid), highest dose of arecoline and arecaidine was inducing highest genotoxicity. However, it was observed that the lowest dose used for arecoline N-oxide is much more toxic than that of arecoline. N-methyl nipecotic acid was showing the minimum level of genotoxicity.
(VI). In the experiment undertaken, three different doses (20, 50 and 80 mg/kg/bw) of areca nut extract, sadagura extract and areca nut with sadagura extract had potential genotoxic effects in somatic as well as in germ cells. In all the three cytogenetic assays (chromosomal aberration, micronucleus, and sperm head abnormality) and total sperm count studied, it was found all the three extracts tested induced genotoxicity to a highly significant level (P<0.001). A time dependent decreased in genotoxicity was also observed in case of chromosomal aberration and micronucleus assays after 24 and 48 hour studies. It is also clearly observed that out of the three extracts, the highest dose of areca nut with sadagura extract showed maximum level of genotoxicity as compared to areca nut extract, and sadagura extract treated alone.

(VII). Arecoline, arecaidine, arecoline N-oxide and N-methyl nipecotic acid have a significant increasing effect on Lipid Peroxidation level in liver tissue of mice. It was very interesting to note that arecoline (20 mg/kg bw) when administered i.p induced Lipid Peroxidation which is significantly high for 24 hour (approximately three and half times more as compare to oral route) and 48 hour (P<0.001) and 72 hour (P<0.05) study regimes. All the tested chemicals after 24 hour exposure induced dose dependent increase in the Lipid Peroxidation level. The highest dose (80 mg/kg bw) was showing a significant increase (p<0.001) in Lipid Peroxidation. A time dependent decline in Lipid Peroxidation level was also observed. When vitamin C was pre-treated, LPO was significantly decreased, indicating the role of vitamin C as a strong antioxidant.
(VIII). In the experiment undertaken, three different doses (20, 50 and 80 mg/kg/bw) of areca nut extract, sadagura extract and areca nut with sadagura extract have a significant increasing effect on Lipid Peroxidation level in mice. All the tested extracts after 24 hour exposure induced dose dependent increase in the Lipid Peroxidation level. The highest dose (80 mg/kg bw) of extracts was showing a significant increase (p<0.001) in Lipid Peroxidation level. However, it was interesting to note that when mice pre-treated with vitamin C for five days were treated with extracts, there was mark decreased in Lipid Peroxidation level indicating the active role of vitamin C as a strong antioxidant in areca nut and sadagura alone or in combination induced oxidative stress.

(IX). Areca nut and sadagura extracts treated alone or in combination have a strong reducing effect on the Mean Survival Time of Dalton’s Lymphoma bearing mice. It is clearly indicted in the results, out of the three different extracts used, the areca nut with sadagura extract (80 mg/kg/bw) oral administration for consecutive fourteen days followed by DLA transplantation showed maximum decreased in mean survival time as compared to areca nut and sadagura extracts treated alone, indicating more toxic when consume in combination. There was a mark decrease in mean survival time of DLA bearing mice which was statistically significant at P<0.001. The average body weight was found to have a significant increase (P<0.001) in cancer control (only Dalton’s Lymphoma Ascites) group. However, it was observed that there was decreased in the average body weight but not statistically significant when compared with the cancer control group after
extract treatment. It is clearly indicted in the results, out of the three different extracts used, the sadagura extract (80 mg/kg/bw) showed maximum packed cell volume compared to other treatment groups. Areca nut extract showed least tumor growth in terms of packed cell volume compared to all other extracts in their equal doses.

Based on the above findings, it can be concluded that areca nut alkaloid arecoline and its metabolites, (arecaidine, arecoline N-oxide and N-methyl nipecotic acid) and areca nut and sadagura extracts are highly genotoxic and can induced Lipid peroxidation, which can generate reactive oxygen species causing damage to the cellular biomolecules and genome of the organism leading to various adverse outcome. It is clearly find out from the present work that arecoline N-oxide is most potent in inducing genotoxicity among all the three metabolites tested and the parent compound arecoline itself. This indicates the possibility of formation of intermediate, more toxic arecoline N-oxide of during metabolism of arecoline which causes genotoxicity as well as may play important role in inducing DNA damage. It is also possible that a process called metabolic inter-conversion takes place in vivo which enhance the retention time of the parent compound before complete metabolism and urinary excretion. The arecoline N-oxide may play a very crucial role in arecoline induced toxicity leading to cellular transformation among people with betel nut chewing habit. Flavin monooxygenase enzyme activity is significantly playing role arecoline metabolism. It is possible that the damage induced by arecoline and its metabolites are due to DNA adduct formation, oxidative stress, as well as the genotoxic potentialities of individual compounds acting together playing a
role in tumor formation in areca nut chewers. In the present study, all the compounds/extracts tested, induced dose dependent increase in sperm head abnormality, indicating their carcinogenic potential which causes point mutation in Y-chromosome in testicular DNA resulting into the formation of abnormal sperm head or change in sperm morphology. Present findings bring a message to millions of betel quid chewers that betel quid chewing is harmful to the gonadal functions which ultimately have an adverse effect on the human gene pool. Therefore, in the light of the present findings, it can be concluded that consumption of areca nut and sadagura are harmful in inducing genotoxicity, as well as become more harmful and genotoxic when consumed in combination. Exposure of areca nut alone or in combination with tobacco exhibit a potent carcinogenic activity, therefore, it becomes necessary to ban areca nut and tobacco chewing products in market and prevent the youth particularly from its habitual consumption.

From the findings of the tumor studies using Dalton's Lymphoma Ascites model, it can be concluded that the areca nut, sadagura and areca nut with sadagura extracts treatments was not able to inhibit tumor cell growth and decreases the packed cell volume. Furthermore, treatment with these extracts reduces the survival time significantly and a decrease in body weight was observed in exposed mice. These results could indicate either a direct cytotoxic effect of the extracts or indirect local effect, concerning macrophage activation and vascular permeability inhibition.

The present findings reveal the involvement of reactive oxygen species in the induction of Lipid Peroxidation by arecoline and its metabolites which is in agreement with the already reported work. Oxidative stress is known to induce
apoptotic cell death via p53 in several cells and the involvement of stress-responsive signaling pathways that could be a possible mechanism of arecoline action leading to inflammatory oral diseases, including oral submucous fibrosis. It is clearly understood from the experimental results that the supplementation of antioxidant, vitamin C can minimize the cellular injury induced by lipid peroxidation products to a remarkable level. Vitamin C has the ability to sequester the singlet oxygen radical, stabilize the hydroxyl radical, and regenerate reduced vitamin E back to the active state. These functions work to halt peroxidation of cellular lipid membranes. Therefore, the experimental results proved beneficial to consume vitamin C rich diet for those persons who chew or consume areca nut and/or sadagura for a long period of time. Vitamin C application in right dose and for proper duration can minimize and mitigate the harmful side-effects of areca nut alkaloid and metabolites on the normal cells and tissues of the body. So, vitamin C should be included among the routine balance diets. It is widely distributed in fresh fruits and vegetables.