CHAPTER VIII
SUMMARY AND CONCLUSION

Toddy is the major country liquor widely consumed in Kerala. Its production in the state is not competent to meet the demand and this leads to its illicit production and adulteration. The adulterated toddy is devoid of many nutritional components present in natural toddy. Because of its low intoxicating effects so many external intoxicating agents like psychotropic substances are added. These substances are reported to have many neurological effects apart from the effect on other systems of the human body.

Considering all these facts, a time dependent study on the change of physical and chemical parameters of natural toddy and the major constituents of adulterated toddy were conducted. The synergetic effect of toddy and commonly detected adulterants namely diazepam, phenobarbitone and chloral hydrate were studied in experimental animals and finally a cell line study using normal liver cells were also conducted.

For the study 80 samples of fresh toddy were collected from the toddy tappers directly, which included coconut toddy, choondapana toddy and palm toddy from Kottayam, Ernakulam, Trichur and Palakkad districts in the state of Kerala. Analyzed the alcohol percentage from the time of collection at an interval of 6 hrs. Odour was noted at various dilutions at the same intervals. Shop toddy were collected from the licensed shops in Kottayam, Ernakulam, Trissur and Palakkad districts from where adulteration was reported frequently. Out of 80 shop samples 24 were found to be adulterated in different ways. The adulterated toddy samples were further analyzed for various physical and chemical parameters.

The natural toddy and adulterated shop toddy samples were analyzed for physical parameters such as odour, turbidity, percentage transmission of light through supernatant liquid and selected chemical parameters like ethyl alcohol content, pH, titratable acidity, ash content, alkalinity of water soluble ash,
phosphorous, potassium, sodium and ascorbic acid in an interval of 6 hrs up to 48 hrs. The shop toddy samples were analyzed for commonly detected adulterants. When comparing the turbidity and percentage transmission of light in natural and adulterated toddy samples, the natural toddy samples showed high turbidity compared to adulterated toddy. The percentage transmission of light is high in adulterated toddy samples than in natural toddy samples. Turbidity is a measure of the degree to which a liquid loses its transparency due to the presence of suspended particles. The more total suspended solids in the medium the higher the turbidity. Natural toddy contained more suspended particles than adulterated toddy which accounts for its high turbidity and less transparency. Natural toddy has a strong odour up to 50% dilution whereas adulterated toddy has medium odour even in 25% dilution.

Ethyl alcohol contents of various toddy samples revealed that adulterated samples contain maximum alcohol percentage compared to natural samples. Added alcohol may be the reason for the high alcohol content of adulterated toddy. The maximum ethyl alcohol percentage obtained for natural toddy samples in the present study is not in terms with the Kerala Abkari Shops (Disposal)Rules,2002.

The pH of toddy samples decreases with increase in time and titratable acidity increases with the time in natural toddy samples. Increase in acidity may be the reason for the decrease of pH with time. In shop toddy samples such a study could not be done since the time of collection of the samples were not known.

Ash content and alkalinity of natural samples are much higher than that of adulterated samples. Phosphorous, potassium, sodium and ascorbic acid contents are also high in natural samples. Since there was no dilution in natural samples, the above contents were high. Shop toddy were analyzed for detection of adulterants. In the present study diazepam, sodium lauryl sulphate, saccharin and starch were detected in shop samples. Quatitative analysis of diazepam and starch were also conducted.

The toxicological study was conducted with three adulterants commonly detected, on male albino rats of wistar strain weighing 130-150 gm and cell line study in normal liver cells.
It was observed that the natural toddy treated groups showed a steady increase in body weight. The natural toddy used for the study is the fresh toddy which contains minerals, vitamins, ascorbic acid etc which are good for health. Toddy along with diazepam, phenobarbitone and chloral hydrate treated group showed a decrease in weight gain percentage compared to control group and adulterant alone treated groups.

Our study revealed that toddy along with diazepam, phenobarbitone and chloral hydrate caused deleterious effects on rats producing oxidative stress in the liver as a result of the generation of superoxide radicals by ethanol and the metabolism of these drugs. Significantly elevated levels of serum enzymes such as ALT and AST indicate increased permeability and damage and/or necrosis of hepatocytes. The results of the present study demonstrate that toddy adulterated with diazepam, phenobarbitone and chloral hydrate has some synergetic effect as evidenced by the elevation of serum ALT, AST, ALP, GGT and LDH along with a decrease in serum cholinesterase activity. ADH and ALDH in the liver of the rats were elevated in all treated groups.

In the present study total protein and albumin levels in toddy adulterated with diazepam group showed a significant decrease and it was more in group IV compared to other groups. In groups treated with phenobarbitone and chloral hydrate along with toddy also showed a decrease in TP and albumin level compared to control and adulterant alone treated groups. In chronic liver diseases, the serum albumin level is reduced due to protein synthesis disruption in the liver. The liver is the site of albumin and fibrinogen synthesis and also some of the alpha and beta globulins. Decrease in the albumin and total protein content in the treated groups are due to decreased synthesis of albumin and other proteins.

TBARS and CD contents showed significantly increased concentration in the liver and kidney of rats treated with combination of toddy and each adulterant compared to toddy alone treated groups. The enhanced level of thiobarbituric acid reactive substances and conjugated dienes indicate the sustained formation of free radicals.
The body has an effective mechanism to prevent and neutralize the free radical induced damage. GSH content in the liver and kidney of animals treated with combination of toddy and each adulterant showed significant decline compared to toddy alone treated groups. Lowering of GSH level indicates that the toxic effect of oxidative insult are exacerbated, resulting in increased membrane and cell damage.

Glutathione-S-transferase showed an increase and catalase showed a remarkable decrease. Increase in glutathione-s transferase suggests its activation due to oxidative stress.

Histopathological studies, compared to pairfed control and natural toddy fed group demonstrated that combination of adulterants and toddy produced hepatic damage. TEM and SEM analysis were also in agreement with neuro degeneration in groups of adulterant and toddy combination compared with the pairfed control and toddy treated group.

This study established the toxic effect of common adulterants of toddy. So these chemicals were screened for their cytotoxic and apoptotic activities in normal chang liver cell line. MTT assay is an established method of determining viable cell number in proliferation and cytotoxicity studies. In the present study Diazepam(50 µl) alone and in combination of diazepam and natural toddy showed maximum cell death and a marginal difference was observed between different groups but not significant. Phenobarbitone was toxic to rat hepatocytes only at concentrations greater than 2 mM. The percentage proliferation decreases with increase in concentration. Chloral hydrate treated cells also showed cell growth. In combinations the proliferation rate decreased. Here natural toddy produced a remarkable cell proliferation and no cell death was observed in any of the concentrations tried. We expected some sort of toxicity in chang cells treated with shop toddy, instead of that we observed cell proliferations. This indicates that the shop toddy, used do not contain any toxic ingredients showing a trend of avoiding the mixing of diazepam, phenobarbitone and chloral hydrate in shop toddy, corroborating the study of recent samples of shop toddy analyzed which are adulterated with starch or saccharin as detected by analytical methods.

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CONCLUSION

The adulteration of toddy primarily involves mixing chemicals like diazepam, phenobarbitone and chloral hydrate to give sedative and muscle-relaxing effects to the consumers. Synergism by ethanol with the effect of diazepam or phenobarbitone or chloral hydrate on the central nervous system is one possible explanation for the supra-additive or potentiating effects of the ethanol-drug combination. Ethanol is known to inhibit the drug metabolizing enzyme system thus preventing the elimination of lipid soluble material from the body. Generation of large amount of reactive oxygen species due to the synergetic effect of drug-alcohol damages the antioxidant defense system this in turn can impair cellular structure and function.

The unbridled use of diazepam, phenobarbitone and chloral hydrate makes one so addicted to the man-made toddy that if the consumer misses his daily dose, withdrawal symptoms surface so severely that it needs hospitalization. Complete ban of toddy tapping in the state is impractical as it is a threat to people associated with this industry for their livelihood.

The only option to control toddy adulteration is to collect samples, test and punish the suppliers severely if traces of chemicals are found. But there is no uniform test that helps to confirm the presence of all chemicals. So a uniform test method for the detection of adulterants must be developed.