GENERAL DISCUSSION
Function of any organ of the body is intimately connected with its structural integrity. Chemical agents including the drugs and the alkaloids have found to alter the structural integrity more specifically at the subcellular structures of the organ like liver. The effect of arecoline, the chief alkaloid of betelnut has not been studied extensively in this direction; and in the present investigation alteration of activity and level of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose-6-phosphatase and glycogen in rat liver have been investigated after feeding the animal with betelnut mixed diet and after administration of arecoline for a period extending upto 28 days. These parameters are expected to give some reliable informations regarding the basic metabolic functional activity of the liver, and the histopathological changes of the liver after exposure to the betelnut and to arecoline can be correlated with the alteration in the activity and the level of the above parameters. It must, however, be borne in mind that enzyme procedures are tests of cellular integrity rather than function. Electron microscopy is definitely one of the best modern tools for such study and for cytochemical localization of metabolic processes within the liver cells and to recognize subtle changes in the biochemical processes of sub-cellular components but lack of facility has prevented to extend the present study to that extent.
In the present investigation significantly elevated levels of alkaline phosphatase, AspAT and AlaAT have been noted both in the groups of animals fed with BN mixed diet (Table 10, 11, 18, 19, 21, 22; Fig. 12, 22, 23) and intraperitoneal administration of arecoline in dose of 1.0 mg. and 2.0 mg. (Table 12, 13, 24, 25, 26, 27; Fig. 13, 24, 25). However, a significantly low levels of AspAT and AlaAT have been noted in the liver of the group of animals fed with BN mixed diet for 28 days (Table 18, 19, 21, 22; Fig. 22, 23). Lowering of both the aminotransferase started earlier in the groups of animals receiving the arecoline intraperitoneally (Table 24, 25, 26, 27; Fig. 24, 25). In the BN mixed diet fed animals the levels of glucose-6-phosphatase were not altered up to 28th day of the experiment (Table 30, 31 and Fig. 30), but there was significant reduction in the liver glycogen content in all the treated groups of both the experiments (Table 36, 37, 38, 39; Fig. 38, 39). The enzyme glucose-6-phosphatase was found to be significantly increased 14 days after exposure to the alkaloid (Table 32, 33 and Fig. 31).

Along with the significant alteration of the liver enzyme patterns and liver glycogen content, discrete type of structural changes in the liver cells of the treated groups of the experiments have also been noted (Fig. 42 to 45, and Fig. 47 to 59). The changes in groups of animals receiving intraperitoneal administration of arecoline started earlier
than the groups of animals fed with BN mixed diet. Infiltration of the lymphocyte in the portal tract and periportal area of the lobules associated with hyperplasia of Kupffer cells and isolated appearance of pyknosis and karyolysis were seen in the same arecoline treated groups.

The hydropic and other changes of liver cells were more pronounced and started earlier in the group of animals receiving 2.0 mg. of arecoline intraperitoneally and thus the structural changes of the liver appeared to be dose and duration dependent.

In the present study on changes in the liver of rats fed with betelnut, a suspected carcinogen, the changes in the studied parameters are reflections of the response of liver to betelnut compounds and arecoline.

The basal level of the alkaline phosphatase in rat liver though very low, but easily inducible by various stimuli of wide variety including alkaloids (Wilfred, 1977; Ikehara et al. 1978; Selvakumar and Wilfred, 1984; Misumi et al. 1986; Watanabe et al. 1986), hepatotoxic chemicals and substances (Robertson et al. 1949; Greenstein and Echenbreunur, 1954; Bodansky, 1961; Shivanandappa and Krishnakumari, 1981). Hormones and steroids (Kaplan and Righetti, 1970; Pekarthy et al. 1972; Baker et al. 1973; Wilfred and Rao, 1976, 1977; Mary and Rao, 1981) are also capable of inducing alkaline phosphatase of liver. It is also reported that there is relationship
FIG. 60: Showing liver ALP, AspAT, AlaAT, glucose-6-phosphatase and glycogen patterns of the experiment-I; values are expressed in percentages taking the values of Control animals as 100 percent.
between increase of alkaline phosphatase and transport of materials across cell membrane (Bodansky, 1934; Glickman et al. 1970; Hirano et al. 1985).

Alkaline phosphatase in mammalian cells is a membrane bound glycoprotein and is a marker enzyme for the plasma membrane. It has been reported that alkaloids have direct action on membranes and interaction of alkaloids with membranes caused increase alkaline phosphatase activity in the cell (Wilfred, 1984; Misumi et al. 1986). The close association of alkaline phosphatase and phospholipids in membranes, and on the basis of Neri's report (Neri, 1971) that in biological system, arecoline shows binding reaction with tissues, it may be possible that binding of arecoline with membrane might result in alteration in its surface topography which may act as a trigger for increased activity of the enzyme.

Many investigators have reported (Ikehara et al. 1978; Oda and Ikehara, 1981) the inhibitory effect of alkaloids on the transport of the induced enzyme to the cell surface caused accumulation of the enzyme; this may also explain the mechanism of increased activity of alkaline phosphatase by betelnut compound and alkaloid arecoline on rat liver.

The cAMP induced increase in hepatic alkaline phosphatase resulting from inhibitory effect of alkaloid on phosphodiesterase as reported by Wilfred (1977), Ikehara et al. (1978), Selvakumar and Wilfred (1984) also is one of the mechanism of increase of the activity of the enzyme by various
FIG. 61: Showing liver ALP, AspAT, AlaAT, glucose-6-phosphatase and glycogen patterns of the experiment-II (1 mg. arecoline); values are expressed in percentages taking the values of control animals as 100 percent.
alkaloids and may be also a possible mechanism of increased alkaline phosphatase noted in the present investigation after administration of arecoline.

Weber (1963) suggested that under the influence of damaging agent, liver alkaline phosphatase increases, and may be due to disturbance in excretion of the enzyme in the face of necrosis. Phelam et al. (1971) reported disturbance in the transport function of hepatocyte or of the biliary tree causes increased amount of hepatic alkaline phosphatase resulting in increased serum alkaline phosphatase activity. It can also be noted that in the present study the highest activity of alkaline phosphatase in the liver was observed on the 14th day in the animals receiving 2.0 mg. of arecoline (Fig. 62) along with some necrotic changes in the liver tissue; whereas in animals fed with betelnut diet the highest activity was observed on 14th day (Fig. 60) which was lower than the activity observed in the animals receiving arecoline; and the alkaline phosphatase activity on the 28th day was lower than the 14th day and at the same time, it may also be noted that in this group necrotic change was not observed in the liver tissue. It is yet to be established the amount of arecoline needed to produce liver necrosis. However, it may be postulated that the quantity of betelnut, fed to the animals, was not sufficient to bring the cellular changes in the liver to that level to influence the level of the enzyme as observed after administration of arecoline intraperitoneally.
FIG. 62: Showing liver ALP, AspAT, AlaAT, glucose-6-phosphatase and glycogen patterns of experiment-II (2 mg, arecoline); values are expressed in percentages taking the values of Control animals as 100 percent.
Of the enzymes providing a link between protein and carbohydrate metabolism, aminotransferases or transaminases are important representatives. The primary role of Asp AT and Ala AT is basically the reversible transfer of an amino acid to a ketoacid. In the present study alteration in the activity of these transaminases in the livers of the animals during the different phases of the experiment, lead to probe the problem in relation to the alteration in the metabolism within the hepatocytes. To review the different factors resulting in altered activity of these transaminases or aminotransferases in liver, it can be noted that many workers such as Chang and Ho (1979); Miranda et al. (1981); Kloss et al. (1982) reported increased activity of amino transferases i.e. Asp AT and Ala AT in animals exposed to different plant alkaloids like pyrrolizidine alkaloid, morphine, cocaine supporting that these plant chemicals have some effects either directly or indirectly on the activity of these enzymes.

One of the primary conditions of alteration in the activity of these enzymes is a state of typical gluconeogenic condition where gluconeogenic hormones play a crucial role (Gavasto et al. 1957; Rosen et al. 1958; Harding et al. 1961; Segal et al. 1962). In this line Schepartz (1973) was of the opinion that the altered activity of the enzymes is an effect rather than cause of the acquired gluconeogenic condition of the cell. Action of alkaloid arecoline and betelnut compounds on the functional activity of the endocrinal axis has been
though studied earlier by Houssay and Molineli (1925,1926), Schlegel (1939), Sirsi et al. (1966), Gurin et al. (1969), Gurin (1971), but not enough to explain changes noted in the present investigation in terms of endocrinal functions.

Another important parameter related with the activity of the enzymes is the protein and nucleic acid metabolism. Betelnut aqueous extract and its constituents; polyphenols, tannins, arecoline have shown to have effect on protein and nucleic acid metabolism (Shivapurkar et al. 1978, 1979). Tannic acid, a component of betelnut found to decrease nucleic acid and protein content in the liver of Wistar rats (Korpassy, 1961). Amino acid availability regulate the rate of protein synthesis and amino acids are synthesized from nucleic acids. Shivapurkar et al. (1978) reported that betelnut aqueous extract, its polyphenolic fraction, tannic acid and arecoline have subtle toxic effect on nucleic acid and protein metabolism. Hence it can be suggested that the decreased activity observed on 28th day of feeding the diet, in case of the two enzymes that catalyzes transaminase reaction may be manifestation of the subtle toxic effect exerted by various betelnut constituents on amino acid and protein synthesis.

However, at this stage, it can be remarked on the basis of the nature of alteration in the activity of these enzymes during the period of the experiment, that there was an initial increase in the activity of both the enzymes followed by decline to the apparently normal level of both the
enzymes in the animals exposed to different doses of arecoline (Fig. 61,62). But with the animals fed with betelnut diet there was significant decline in activity of both the enzymes on 28 days of feeding (Fig. 60). Considering these observations it can be summarized that betelnut compounds and its active principle arecoline effects the liver, with an initial increase in transaminase activity followed by reduced activity of liver transaminase or amino transferase (Fig. 60,61,62). And at the same time it can also be stated that the decline in activity after the initial increase was more related to the duration of exposure rather than the intensity of stimulus. At this stage it can be considered that on exposure to betelnut compounds the liver is responding with an increased pace of its protein synthesizing machinery, which may be a part, of the rearrangement of the protein picture in the hepatocytes, necessary for maintenance of a newly acquired functional state of the cell induced by the active agent present in the betelnut or some of its metabolic products. Further, it can not be denied that the activity of transaminase is essential in the process of protein rearrangement as in that phase of cellular activity, availability of a balanced intracellular amino acid state is a crucial factor, much contributed for its maintenance by the process of transamination. The ultimate decline towards the end of the experiment on 28th day, may signify end of the protein rearrangement or more likely a failure in the
protective response of the cells. However, it can also be viewed with another perspective that the final decline is a simple rebound phenomenon associated with rearrangement to its original state after overcoming the response to the stimulus. At the same time simultaneous effects of the different mechanisms can not also be ruled out. Because, with lower intensity of stimulus in the animals fed with betelnut for a longer duration of 28 days the histopathological changes reflecting structural integrity of the cells were not much deviated from the control group. Whereas, in animals exposed to relatively higher doses of betelnut compound in the form of arecoline for a much shorter duration of only 14 days, though aminotransferase activities were apparently normal after the initial increase, the histopathology revealed much alterations in the structural integrity in the cells in the form of necrosis, specially in groups receiving 2.0 mg. of arecoline. So, considering these observations, simultaneous presence of any of these activities cannot be entirely ruled out.

In the present study, another enzyme playing an important role of glucose homeostasis in liver, working as a deciding factor for the exit and utilization of liver glucose is the glucose-6-phosphatase. It is observed that there was no significant alteration in the glucose-6-phosphatase activity in liver of rats fed with betelnut mixed diet throughout the period of experiment (Fig. 60) whereas in
animals treated with 1.0 mg. and 2.0 mg. of arecoline daily, the glucose-6-phosphatase activity found to be elevated on the 14th day (Fig. 61, 62). Two important criteria related with the activity of the enzyme are that it acts as a deciding factor for releasing glucose from liver to the periphery and it is an inducible enzyme. The enzyme can be induced by the level of substrate (Hers and Hue, 1983) which may be caused due to condition of stimulated gluconeogenesis as reported by Weber (1963). And it can also be mentioned that in situation for increased peripheral demand for glucose, the adequate activity of this enzyme is the only solution.

Considering this crucial factor associated with the role and activity of liver glucose-6-phosphatase, it can be remarked that betelnut and or its metabolites do not have considerable effects on liver to bring about sufficient changes to initiate induction of glucose-6-phosphatase. However, it may also be mentioned that the apparent and alternate weekly fluctuation (Fig. 60) in the glucose-6-phosphatase activity during the period of experiment may be the indication of a minor imbalance in regulation of glucose-6-phosphatase activity through the regulatory effect of glucose-6-phosphate, super-added by the induction phenomenon to an insignificant level.

However, it can also be noted that in contrast to the above experiment, the major component and an active principle of betelnut, arecoline, or its metabolites have some observable effects on the liver glucose-6-phosphatase activity with sustained
exposure. In light of different mechanisms already discussed in relation to variation in liver glucose-6-phosphatase activity, observation of no significant change in the first week and significant alteration in the 2nd week (Fig. 61,62) may be because of an amplified effect of the same mechanisms. In the first week of the experiment, 1.0 mg. and 2.0 mg. of arecoline administered daily for 7 days may not be sufficient to produce any appreciable change in the enzyme activity specially through the mechanism of induction and at this stage homeostasis is still maintained regarding glycolysis, gluconeogenesis and peripheral demand for glucose, probably through the regulatory effect of glucose-6-phosphate on activity of the enzyme. On the succeeding period, the sustained action of arecoline in this newly adapted state may finally be responsible for the significantly elevated enzyme activity brought about by the induction phenomenon initiated by sustained higher glucose-6-phosphate content in hepatocytes through the usual process. Weber and Cantero (1955) remarked that "the increased glucose-6-phosphatase activity might be considered as an adaptive response of the organism".

In the present investigation, the amount of liver glycogen was observed to be significantly low on the 14th and 28th day in the animals fed with betelnut mixed diet, showing a fluctuation in the liver glycogen content during the period of the experiment (Fig.60). In the animals exposed to arecoline with varying daily doses, the liver glycogen content was
significantly reduced from 7 day onwards up to the end of the experiment on the 14th day where the effect was more pronounced with the daily dose of 2.0 mg. of arecoline (Fig. 61,62).

Carbohydrate, in the form of glycogen in liver acts as the buffer in the maintenance of blood glucose within a narrow physiological range. At any given instant the amount of glycogen present in liver reflects the state of total carbohydrate metabolism at that instant.

The mechanism responsible for maintenance of liver glycogen are complex with intricate interrelationship within themselves and with many other factors. In the wide array of factors concerned with liver glycogen content, availability of glucose and demand for utilization of glucose, are the two primary determinants which will dictate the dynamics of glycogen metabolism by enzymatic, hormonal and neural influence on the target cells.

On the light of the observation in the present study it can be assumed that betelnut and arecoline have produce some alterations in the process, involved with maintenance of liver glycogen balance either directly or indirectly. Similar reports on the effect of betelnut constituents like polyphenol, tannin (Korpassy, 1961; Camp et al. 1967; Shivapurkar et al. 1978), arecoline (Kiyohara, 1931), are available from the studies of different workers. Alkaloids from various plants have shown to effect liver glycogen
content in different test animals (Kobayshi, 1938; Hambresin and Schepens, 1946; Diamant, 1958; McLean, 1970; Saul et al., 1985) also.

Regarding the mechanism or mechanisms involved in production of the observed effects by these group of compounds and most specifically by compounds present in betelnut and by arecoline which was specifically used in the present investigation, it is quite difficult to remark on the actual role played by these compounds as it has been already mentioned that the total glycogen content of liver at a given instant is susceptible to the diversity of the regulated mechanisms of carbohydrate metabolism as a whole. In view of this remark if the mechanism involved with the synthesis and breakdown of glycogen are considered to be effected by the betelnut compounds and/or arecoline, then the observation in this experiment will indicate a negative glycogen balance in the liver of the experimental animals suggesting more probability of involvement of the mechanisms associated with decrease in its synthesis in the liver. At the same time at this stage although the involvement of the mechanisms related with glycogen breakdown cannot be ruled out, it can be easily stressed that at least, these mechanisms would have to be either kept unaltered or enhanced.

The different factors like hormone, neural and enzymic influences acting on hepatic glycogenolysis, originating from diverse locality of metabolic regulatory
mechanisms, ultimately produced the target effect, by their influence or action on the enzymes related with liver glycogenolysis. Liver phosphorylase is the key enzyme for the initiation of hepatic glycogenolysis. Factors which have influence on liver phosphorylase are hormone and autonomic nervous system (Shimazu and Amakawa, 1968a, 1968b; Shimazu, 1971; Matsushita et al. 1979; Matsushita and Shimazu, 1980), they modulate phosphorylase activity by balancing the activity of phosphorylase a and phosphorylase b. Another factor related with the activity of phosphorylase is cAMP. The present observation of the effect of arecoline and betelnut on glycogen suggest the necessity of further work on phosphorylase enzyme.

Houssay and Molineli (1925, 1926); Rom and Serduck (1927) reported that arecoline causes secretion of epinephrine; Sirsi et al. (1966) have shown that arecoline and the polyphenols of the arecanut to potentiate the epinephrine activity. Epinephrine secreted from adrenal medulla may act directly on the liver or enhance glucagon secretion (Porte and Halter, 1981). The reported enhance secretion of glucagon will act on the hepatic glycogenolysis via cAMP. cAMP activity reported to be directly enhanced by many alkaloids (Robinson, 1968; Peach, 1972; Wilfred, 1972). So, the direct action of betelnut compounds/or arecoline on adenylate cyclase activity may also be suspected resulting
in the observed changes/or may be thought as one of the process involved with observed changes.

Hepatic glycogen balance is dependent on glycogenesis whose stability is determined by two factors, super-threshold intracellular concentration of glucose-6-phosphate for activity of phosphoglucomutase and reduced peripheral demand for glucose. Of these two primary factors the pace of hepatic glycogenolysis is more sensitive to the reduced peripheral demand as the threshold concentration of glucose-6-phosphate in favour of glycogenesis can be maintained only after overcoming the demand for glucose in the periphery.

In the present investigation observation of a fluctuating and gradually diminishing liver glycogen content in the animals fed with betelnut mixed diet (Fig. 60) and reduced liver glycogen content in the animals treated with different doses of arecoline (Fig. 61, 62) hints at impairment in the balance between the two critical factors governing hepatic glycogenolysis.

Without any idea regarding the phosphoglucomutase activity in these experimental animals it is rather difficult to comment on the contribution of the glycogen synthesizing enzymes on the observed changes on hepatic glycogen level in this experiment. However, it may be recalled at this situation that the glucose-6-phosphatase activity was without any significant change in the animals fed with betelnut mixed diet throughout the period of the experiment and it was
significantly enhanced in the animals receiving different daily doses of arecoline on the 14th day without any significant deviation from the Control group on the 7th day. In consideration of these findings, it can easily be assumed that there was very low probability of an adequate level of glucose-6-phosphate sufficient to initiate or maintain the process of glycogen synthesis. In this situation, it may also be mentioned that in the present investigation when the relationship between the level of glucose-6-phosphatase and liver glycogen in a group have been tried in animals treated with arecoline and also in animals fed with betelnut diet, the correlation co-efficient value (Table 40) found to be very low indicating non-existence of any relationship between these two parameters. Weber (1959) also mentioned that there is no necessary correlation between glucose-6-phosphatase activity and glycogenesis.

It is well established by works and reports of many workers that arecoline has direct stimulatory effect on motor end plates (Robinson, 1968; Goodman and Gilman, 1985) and central nervous system (Henderson and Roepke, 1937; Peeiffer and Jenney, 1957; Leslie, 1965; Belesin et al. 1974; Tsujimoto et al. 1975). These observations suggest that under the influence of arecoline there might be more peripheral utilization of glucose resulting in stimulation of glycogenolysis.

It may also be noted from the histopathological observation from liver of experimental animals, that there
was appearance of hydropic degeneration at all stages of the experiment indicating an impaired electrolyte balance in the hepatocytes. It was reported by many workers (Williams and Carter, 1965; May and Carter, 1967, 1970; Avrunin and Carter, 1970) that arecoline causes marked natriuresis and chlorouresis by its direct action on the renal tubular cells causing impairment in the sodium readsorption. On the basis of this report it may also be thought that by similar type of activity of arecoline on the sodium related symport mechanism of the glucose on the intestinal brushborder, the glucose absorption may be diminished and thus contributing continued negative balance with impairment in the exogenous source of glucose to the animal.

In the present study "changes in the liver of rats fed with betelnut a suspected carcinogen", on consideration of all the observations, it can be suggested that betelnut as a whole or some of its active principles either in native form or metabolites, have some significant effect on the metabolic as well as structural state of the liver in the experimental animals as reflected by alterations in the level of alkaline phosphatase activity, AspAT activity, AlaAT activity, glucose-6-phosphatase activity, and glycogen content within the period of the experiment which is supported by a similar but somewhat amplified version of the same effect in the group of animals directly exposed to arecoline.
the active principle of betelnut, through the intraperitoneal route. Although it is very difficult to pinpoint any mechanism or mechanisms involved, which brought about the observed changes; from the foregoing discussion, it is apparent that the liver, in this experiment is trying to maintain a newly acquired homeostatic state. For a more precise view on the problem, expanded work involving other parameters related with the problem are to be probe.