DISCUSSION

In the present finding it was found that CLGG is the ideal matrix for the purification of \textit{Abrus} lectins. In the affinity evaluation study, it was found that about 35\% lectin was obtained from 20 mg of total protein loaded on to the CLGG affinity column. Purification of lectins using Sepharose-4B and AT-Sepharose-4B matrices resulted in a recovery of 15 and 20 \% lectins out of the total proteins (20 mg) loaded. In the earlier, reports it was found that the CLGG was the best affinity matrix when compared to other affinity matrices for the isolation of several galactose-specific lectins. It is interesting to note that CLGG has a high binding capacity for both \(\alpha\) and \(\beta\) anomer specific lectins (Appukuttan \textit{et al}, 1977; Kumar \textit{et al}, 1982). This may be due to the increased expression of galactose residues in the gel compared to those in Sepharose. Moreover, the cost of CLGG preparation was found to be very economical i.e. 100 g matrix costs US$ 0.25 (about 10 Indian rupees) when compared with other commercial affinity matrices.

Several methods have been followed in the purification of lectins from \textit{A. precatorius} and their properties were studied (Table 1). The physico-chemical properties of the purified lectins from different laboratories are much similar, but different names were adapted for each lectin isolated by various procedures. Olsnes \textit{et al}, (1974b) isolated a toxin and an agglutinin on DEAE-cellulose. Further agglutinin resolved into two peaks on CM-cellulose column chromatography which were not characterized. Wei \textit{et al}, (1974) isolated two abrins, abrin A and C for the first time by different ion-exchange chromatographies. Abrin A was further characterized by Hermann and Behnke (1981) who showed that Abrin A contains three isoforms. Roy \textit{et al}, (1976) reported three isoforms for \textit{Abrus} agglutinin, while Olsnes \textit{et al}, (1974)
and Wei et al., (1974b) reported only one. Later, Lin et al., (1978) reported four isobrins and one agglutinin. Recently, Hegde et al., (1991 and 1992) isolated three abrins and two agglutinins from A. precatorius by lactamyl-Sepharose affinity chromatography followed by DEAE-Sephacel ion-exchange chromatography. In the present study, one toxin and one agglutinin could be purified to homogeneity from all the three Abrus species by using CLGG-affinity chromatography followed by DEAE-Sephacel ion-exchange chromatography.

The SDS-PAGE of the abrins in the presence and absence of β-mercaptoethanol revealed that the abrins consist of two different molecular weight subunits, A-chain and B-chain, which are linked by a sulphhydryl bond. The SDS-PAGE analysis of agglutinins revealed single protein and multiple subunits in the absence and presence of β-mercaptoethanol, respectively. The abrins and agglutinins agglutinated human erythrocytes of all types and their binding to human erythrocytes was inhibited by galactose and galactose containing sugars suggesting that both the lectins bind to the same or similar sites on the blood cells or erythrocytes. On the other hand, the binding of Abrus lectins to rabbit erythrocytes could not be inhibited by any of the carbohydrates tested. This is consistent with earlier report on A. precatorius seed lectins (Olsnes et al., 1974b). Among the carbohydrates tested, β-lactose and Me-β-Gal were the most potent inhibitors of the lectins (Table 5). The data presented clearly shows that the primary requirement for carbohydrate interaction with Abrus lectins is the presence of an axial hydroxyl group at the C-4 position. This is borne out by the fact that the sugars recognized by these lectins such as Me-a-Gal, Me-β-Gal, galactose, lactose and fucose which have an axial hydroxyl group at the C-4 position. Both the lectins, clearly, prefer the P-anomer of galactose, since it
binds Me-β-Gal with twice greater affinity than Me-α-Gal. The low specificity of fucose as compared to galactose indicate that the C-6 hydroxymethyl group in the galactose configuration contributes positively to the binding. Unlike galactose, which has a CH₂OH moiety attached at the C-5 position, fucose has a methyl group in the corresponding position. This implies that fucose denies any favorable hydrogen bonding interactions involving the hydroxyl of the primary alcohol moiety that might exist in lectin-Gal complexes. This is borne out by the fact that this sugar is a weaker ligand for the lectins than galactose itself. Among the disaccharides too, those with β-configuration for the terminal non-reducing galactose residues served as better ligands. p-Lactose binds with approximately the same affinity as Me-β-Gal and 8 times higher than melibiose (Galα1,6Glc) for abrins and agglutinins of A. precatorius and A. fruticulosus. P-Lactose binds with the affinity approximately 8 and 16 times better than melibiose (Galα1,6Glc) as in the case of y-abrin and APSA respectively. The trisaccharide, raffinose is weaker than galactose. This is consistent with the lectins showing higher preference for the p-anomer of galactose, because the galactose moiety at the non-reducing terminus in raffinose (Galα1,6Glcβ1,2Fru) is in the α-configuration. Comparable binding of Me β Gal and lactose clearly indicates that the Abrus lectins preferentially binds the p-anomer of galactose. It was also reported that in galactose specific lectins the hydroxyl groups at C-4 on sugars are the primary binding sites for their recognition by lectins (Khan et al., 1981a and b; Shaanon et al, 1991; Komath et al, 1996; Elgavish and Shaanon, 1997).

Studies on the toxicity of the lectins to rats and dose dependence were performed. The LD₅₀ dose was found to be 2-5 fig/Kg for abrins and 151-160 μg/Kg for agglutinins clearly suggesting that the abrins are toxic proteins. It was already re-
agglutinins clearly suggesting that the abrins are toxic proteins. It was already reported that abrin from *A. precatorius* was a cytotoxic or type II ribosome inactivating protein (type-2 RIP) (Olsnes and Pihl, 1973b; Olsnes *et al.*, 1974a; Olsnes and Pihl, 1982; Endo *et al.*, 1987; Stirpe *et al.*, 1992). Abrin, a type-2 RIP was found to possess N-glycosidase activity and inactivates the 60s ribosomal subunits by cleaving a specific adenine residue from rRNA and thereby arresting protein synthesis (Endo and Tsurugi, 1987). In intact animals, inhibition of protein synthesis most likely accounts for the lethal effect of the abrins.

It was interesting to note from the data of western blots that the p-abrin antibody reaction was strong to A-chain of abrins and high molecular weight subunits of agglutinins. This suggests that the immuno-epitopes are present more on A-chain of abrins and high molecular weight subunits of agglutinins, indicating their structural similarity, which corroborate their functional similarity. A significant immunoreactivity was also found between p-abrin antibodies and agglutinins, which supports the growing evidence that abrins and agglutinins share a certain degree of similarity in their primary structure. The immunological relatedness among these proteins suggested that there might be a unique portion of these proteins that is evolutionarily highly conserved.

The data obtained from two-dimensional electrophoretic analysis of the subunits of abrins and agglutinins of all the species revealed that the subunits of one protein were not shared with that of other protein. Based on this result, it can be predicted that abrins and agglutinins are products of homologous genes expressed independently of one another. Subunits of both the lectins in all the species existed as single forms and no isoforms were found as confirmed by two-dimensional electro-
could be due to post translational modifications including proteolytic cleavage and variable degrees of glycosylation (Strosberg et al., 1986; Hegde and Podder, 1992).

A number of storage proteins from a variety of plant seeds are initially synthesized as precursor polypeptides containing more than one subunit type, and processed post-translationally (Youle and Huang, 1978; Turner et al., 1981; Barton et al., 1982; Brienger and Peterson, 1982; Cray et al., 1982.)-

Lectin distribution in vegetative tissues of 12 day old seedlings A. precatorius was studied using a combination of haemagglutination assay and immunoblot analysis. The relative abundance of lectins in the seed and its concomitant decline after germination is in accordance with the probable role of seed lectins as storage proteins. The results of immunoblot analysis indicate that the leaves and stems contain lectin similar to abrin whereas the cotyledons and roots contain both abrins and agglutinins of seed. This could be the possibility for the enormous difference in the heamagglutinating activity between upper and lower parts of the seedlings. This study on differential distribution of lectins indicates that different tissues for different functions may utilize lectins. The preferential accumulation of abrins in the storage organs is certainly indicative of their possible role in defense against pests and diseases. Resting storage organs and seeds are particularly vulnerable, since they are most attractive to potential parasites and predators, and may lack an active defense system because of their inactive metabolic state. Taking into account the evolutionary adaptation of plants, it can be reasonably argued that they have developed defense system to protect their storage organs and seeds. From this point of view the preferential accumulation of lectins in typical storage organs is certainly indicative.
To evaluate the role of the anti-insect activity of the abrins, studies were conducted on the coleopteran insect *Sitophyllus oryza* and lepidopteran insect larvae of *Corcyra cephalonica*. As the final instar of lepidopteran larvae is the most active stage in its life cycle, the larvae feed enormously and synthesizes large number of biomolecules to be used as reserve energy material during the non-feeding pupal stage. However, when the larvae were fed on diet containing abrins, the insects were died at the pupal stage. All the three abrins were almost similar in their toxic effects on both the coleopteran and lepidopteran species displaying insecticidal property but their mechanism of action is unknown.

An intriguing hypothesis would be that the insecticidal effect of toxin is directly related to the ability of the putative lectin like B-chain to recognize and bind to carbohydrate ligand(s) on the cells lining the midgut wall of the insect. Precedence for such a mechanism comes from the demonstration of specific binding of the lectin *Phaseolus vulgaris* to the epithelial cells of the bruchid beetle (*Callosobruchus maculatus*) mid gut. culminating in the mortality of the insect demonstrating that the mechanism of toxicity was analogous to that mammals (King *et al.*, 1980; Gatehouse *et al.*, 1984). Arcelin-1, is a lectin like protein displays insecticidal activity and protects the seeds from predation by larvae of various bruchids (Osborn *et al.*, 1980). This protein that displays an intrinsic specificity in binding complex glycans and might explain the mode of insecticidal action of Arcelin (Gatehouse *et al.*, 1987; Fabre *et al.*, 1998). Arcelin-4 was found to be toxic to larvae of *Zabrotes aubfasiatus* and its antimetabolic effect was speculated to be due to its indigestibility by gut proteases in the insect as proposed by Minney *et al.*, 1990. Haider and Ellar (1987) proposed mechanism of cytolytic effect of *Bacillus thuringeinsis* crystal 8-endotoxin on
Bombyx mori cell lines. The initial interaction of the toxin with the unique receptor of the host determines the specificity of the toxin following which, cell death occurs by a mechanism of colloidal osmotic lysis. Recent reports by Nagamatsu et al., 1998 also suggest that the toxin act on the brush border membrane by binding to midgut receptor, subsequently breaking its infolding structure and causing cell lysis. Therefore the receptor specificity may be the mode of insecticidal action for abrins following which, cell death occurs by the inhibition of any of the metabolic pathways.

Another possible mechanism of action is that abrins are RIPs and hence might, therefore inhibit protein synthesis of insects eventually leading to their death. This is in corroboration with the result of Gatehouse et al., 1990 who have reported that ricin to be highly toxic to the Coleopteran insects Callosobruchus maculatus and Anthomonomus grandis. Another type-2 RIP a lectin from the bulbs of Eranthis hyemalis (Kumar et al., 1993) was found to be very toxic to larvae of Diabrotica undecimpunctata, a major insect pest of maize. The present study offers evidences, which suggests that the abrins might act as inhibitors of protein synthesis leading to death of insects. The accumulation of toxic lectins in the seeds has been considered to be a protective mechanism (Peumans and Van Damme, 1995). The results support the hypothesis that genes of the legume lectin family encode proteins that function in plant defense against herbivores.