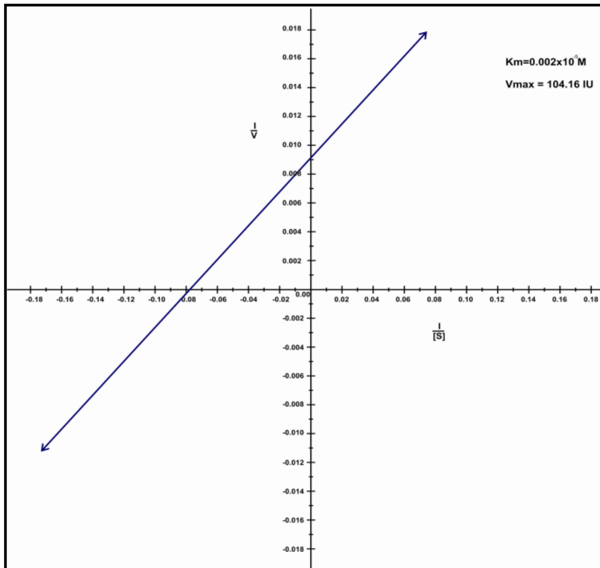
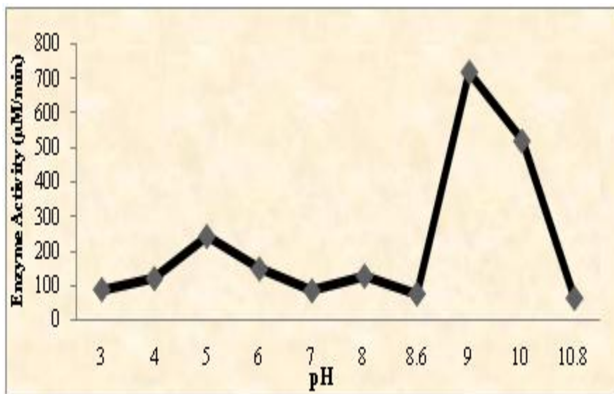
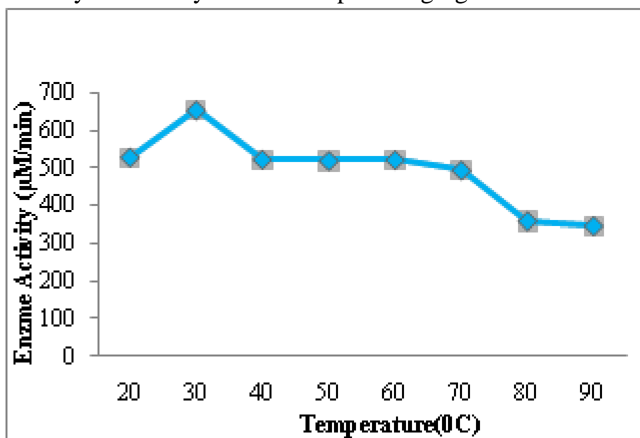


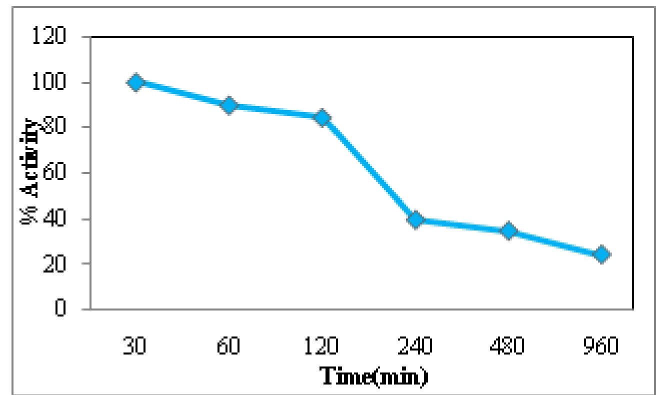
Figure 3: Characterization of L-asparaginase obtained from *A. niger*. (a) The Km value was determined by incubate the enzyme at different concentrations of asparagines



(b) The optimal pH of activity was assessed by measuring the enzyme activity at different pHs ranging from 3 to 10.8



(c) The optimal temperature for activity was assessed by measuring the enzyme activity at different temperatures between 20°C and 90°C



(d) For thermostability test the native enzyme was incubated at optimum temperature at 37°C for different time of up to 960 min.

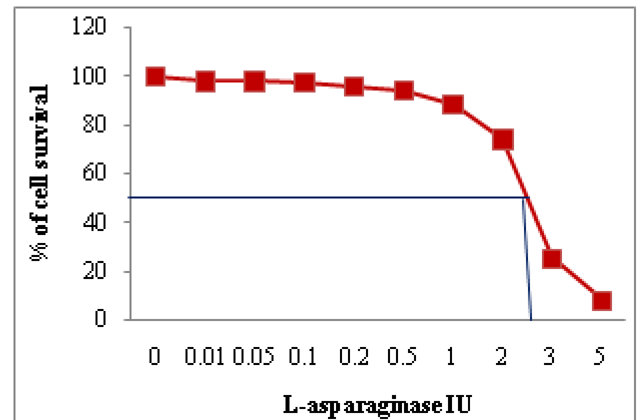


Figure 4: Cell viability of leukemic cells (A431) after treatment with purified L-asparaginase from *Aspergillus niger*

3.2 Cytotoxic Activity

The leukemic cells (A431) incubated with L-asparaginase had their cell viability by 50% in a concentration of 250 µg·mL^{-1} in an incubation time of 72 h, but after this time, the cell viability increased again (**Figure 4**).

4. Discussion

Since the first observations that some L-asparaginases demonstrate anti-leukemic activity, great progress has been made in the therapeutic protocols that combine L-asparaginases with other chemotherapeutic drugs. However, the favorable effects achieved with L-asparaginase in leukemia treatment are accompanied of the undesired side effects as thrombosis, pancreatitis, renal complications, liver damage among others. For this reason, several reports has showed different enzyme formulations and new sources of L-asparaginase included those from eukaryotic organisms, to find less toxic enzymes that also show suitable antineoplastic effects. Our studies of L-asparaginase produced by *A. niger* started determining of the good conditions for its production and the its highest levels were detected if L-proline 2% was the nitrogen source [14] and it is in accordance with the results reported by Sarquis *et al.* [4]. The molecular weight found here for the enzyme was 136 kDa, similar value has been described to *E. coli* L-asparagi- nase, 134 kDa. However, the Km value of native

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L-asparaginase was found to be 2.42 mM, while that from *E. coli* is 0.0125 mM [15]. It shows that the L-asparaginase from *A. niger* has less affinity for asparagine than L-asparaginase from *E. coli* but the L-asparaginase from *A. niger* was capable to inhibit the prolifiration of leukemia cells. According to Panosyan *et al.* [16] the effective deamination of glutamine by L-asparaginase appears to contribute to the decrease of asparagine depletion by depriving the asparagine synthetase of glutamine, the precursor asparagine biosynthesis. Bacterial L-asparaginase, used in therapeutic protocols, has low glutaminase activity but toxicity reactions are attributed to this activity [17, 18]. Herein L-glutaminase activity was not detected in crude enzyme (concentrated or not) produced by *A. niger* even after 60 min of the reaction. This should contribute significantly to diminution of side effects and it may be helpful in clinical practice.

The antiproliferative effects of L-asparaginase produced by *A. niger* was evaluated after 24, 48, 72 and 96 h of incubation of A431 leukemia cell line. This L-asparaginase caused 50% reduction in cell viability after 72 h on the cell line HL-60 and after 96 h on the cell line RS4;11 (**Figure 4**). Interestingly, cell proliferation of HL-60 cell increased after 72 h and this can be associated with multiple adaptive cellular mechanisms. Studies have demonstrated increased asparagine synthetase (AS) expression in cells treated with L-asparaginase. It has been hypothesized that this elevated activity allows these leukemia cells to become resistant to the treatment. Moreover, other adaptive processes may provide a substrate to asparagine synthetase such as aspartate or glutamine, which derive from intracellular and extracellular sources [19,20].

The results of the present study clearly indicate that the L-asparaginase produced by *A. niger* has a molecular weight similar to the *E. coli*, does not present glutaminase activity. Moreover, this L-asparaginase caused antiproliferative effects on A431 leukemia cell line. Altogether, these data prompted further investigations into the L-asparaginase produced by *A. niger*.

5. Conclusion

Phe characterization of the enzyme revealed an optimum at pH 9.0. This property of enzyme makes clear that enzyme produced by *Aspergillus niger* under the present study has effective carcinostatic property, because the physiological pH is one of the perquisites for anti tumor activity. The optimum temperature for L-asparaginase activity was found to be 37^{0}C which is the physiological temperature. This property of enzyme is most suitable for complete elimination of asparagines from the body when tumor patient is treated with L-asparaginase *in-vivo.* Even though the enzyme showed maximum activity at body temperature and physiological pH and its considerable stability over a wide range of *p*H and temperature makes it highly favorable to be exploited as a potent anticancer agent.

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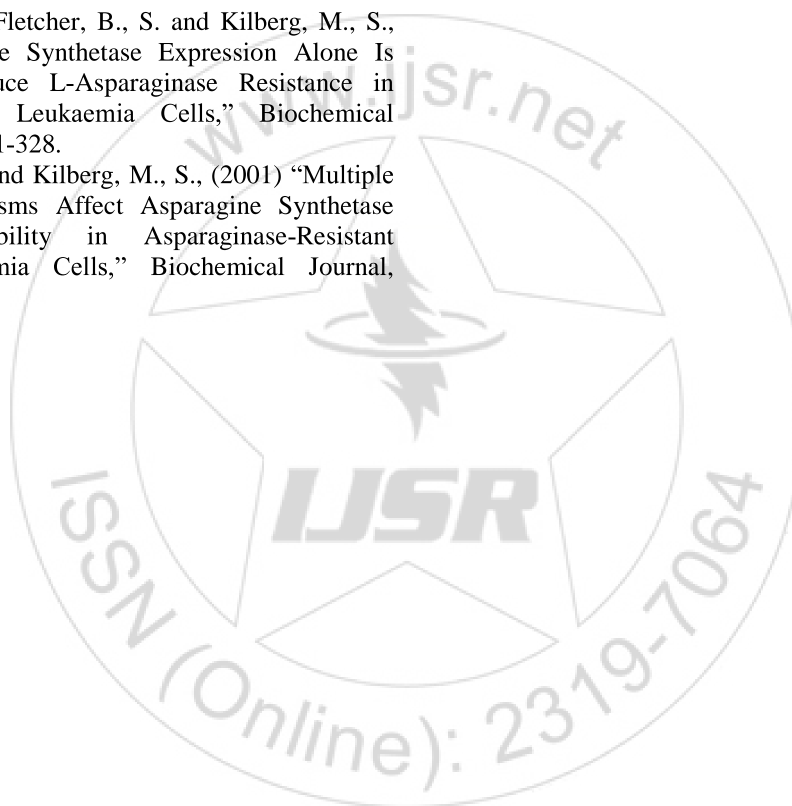

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