Introduction

*Jatropha curcas* L. is a soft-wooded shrub, commonly grown in rural areas in India. The oil of this plant is used for manufacture of various household commodities and industrially useful products. It is also used traditionally for the treatment of sciatica, dropsy, paralysis, rheumatism, dysentery, diarrhea and certain skin diseases (Anonymous, 1959; Desai, 1975; Dymock et al., 1976; Nadkarni, 1976; Agarwal, 1986; Ambasta, 1986).

*Jatropha curcas* L belongs to the family Euphorbiaceae and is distributed in many tropical and subtropical countries. The toxicity of the whole seed from *Jatropha curcas* has been known for a long time. Its toxicity has been attributed to a protein component known as curcin. *Jatropha curcas* is a drought-resistant bush or small tree with spreading branches and stubby twigs that grows to 20 feet high under favorable conditions. *Jatropha* has both male and female plants, which may produce different yields of nuts. The life-span of *Jatropha* may be more than 50 years.

*Jatropha Curcas* has the advantage that not only it is capable of growing on marginal land, but it can also help to reclaim problematic lands and restore eroded areas. As it is not a food or forage crop, it plays an important role in deterring cattle, and thereby protects other valuable food or cash crops. *Jatropha* seeds can be pressed into biodiesel that can be used to run diesel engines, which in turn can drive pumps, food processing machinery, or electricity generators. The bio-oil can also be the basis for soap making. The pressed residue of the seeds is a good fertilizer and can also be used for biogas production.

The leaves and nuts of *Jatropha* are toxic. The *Jatropha* seeds contain a toxic principle, toxalbumin curcin ("curcin" is similar to "ricin", the same toxic protein as present in castorbean *Ricinus communis*) and "diterpine esters" which have been isolated from seeds and roots. Since the seed cake
still contains oil, it also contains the toxic diterpenes, and so can not be used for fodder. Physic-nut oil consists of glycerides of palmitic, oleic, and linolic acids. The bark of the stem contains a considerable amount of chlorophyll, reducing sugars or other reducing substances, saponin, and a small amount of tannin, resin, and a trace of volatile oil. Euphorbin and alkaloid are absent. Sack states that yields a wax, which is a mixture of melissyl alcohol and its melissinic acid ester.

The Jatropha seed contains approximately 40% oil, 18% protein, 33% carbohydrate, 5% fiber, and 4% ash. Leaves show antileukemic activity, contain α amyrin, β sitosterol, stigmasterol, and campesterol, 7-keto-β-sitosterol, stigmast-5-ene-3 β, 7 α diol, and stigmast-5-ene-3 β, 7 β-diol. Leaves contain isovitexin and vitexin. From the drug saccharose, raffinose, stachyose, glucose, fructose, galactose, protein, and oil, largely of oleic- and linoleic-acids, curcasin, arachidic, linoleic, myristic, oleic, palmitic, and stearic acids are also reported. Though all parts of the plant are poisonous, seeds have the highest concentration of the toxin and are highly poisonous. The adverse effects following consumption of seeds result primarily from gastroenteritis.

The clinical symptoms include vomiting, diarrhoea, abdominal pain, and burning sensation in the throat, depression and circulatory collapse have also been reported and are said to be common in children. Human deaths by this plant have not been reported so far though animal deaths can occur. The toxic dose is not known. However, in some instances consumption of as few as three seeds has produced toxic symptoms; while in others, as many as 50 seeds produced relatively mild symptoms.

The toxic and irritant compounds isolated so far from *Jatropha* seeds include β-D-glucosides of sitosterol (Bose, *et al.*, 1961), curcin (Stripe *et al.*, 1976) flavonoids vitexine and isovitexine (Sankara *et al.*, 1971) and the 12-
deoxyl-16-hydroxyphorbol (Adolf et al., 1984). The toxicity of *J. curcas* is considered to be caused by a lectin-curcin (Asseleih et al., 1989).

A toxic protein was isolated from the seeds of *Jatropha curcas* by Felke et al., 1914, and was designated as “curcin”. Curcin was a kind of toxalbumin (Felke et al., 1914), present in very high concentration in the Jatropha seeds. Curcin resemble bacterial toxins in structures and physiological effects. The phytotoxin curcin is a member of the large family of plant ribosome-inactivating proteins (RIPs). Curcin is Type I ribosome inactivating protein, a single chain protein (Barbieri et al., 1993; Stirpe et al., 1976). Many plants contain proteins that are capable of inactivating ribosome and accordingly are called ribosome- inactivating protein (RIP). RIPs are being studied in the biological and biomedical fields because of their unique activity as cell-killing agents. RIP can be classified into three types on the basis of their structure and functions: Type I RIPs are single polypeptide chain with the enzymatic activity with Mw. 28,000- 35,000 and alkaline isoelectric points (pl) of pH 8-10 with or without carbohydrates and can inhibit cell-free protein synthesis, but they are relatively non-toxic to cells and animals; Type II and Type III RIPs are significantly different from Type I RIPs in lectin and enzymatic activity (Stirpe et al., 2004; Park et al., 2004). Ribosome-inactivating proteins (RIPs) existing in many plants are N-glycosidase. RIPs can break the N-glycosidic bond that links the A-4324 to the polyphosphate backbone of the 28S rRNA (Girbes et al., 2004; Peumnas et al., 2001). This makes ribosome unable to bind the elongation factors 1 or 2, consequently arresting protein synthesis (Endo et al., 1987). For a long time the interest in RIP has been focused on developing antitumor drugs that selectively target to tumor cells (Lin et al., 1970).

Antitumor activity is related to N-glycosidase action. Curcin has an obvious antitumor effect. Antitumor activity of curcin was tested by MTT assay. The N-glycosidase activity of curcin was determined by
characterization of R-fragment in gel. A cell-free system, rabbit reticulocyte lysate, was introduced to quantify the inhibitory activity of curcin on protein biosynthesis. The curcin had a powerful inhibitory action upon protein synthesis in reticulocyte lysate with an IC$_{50}$ value of 0.19 (0.11 – 0.27) n mol/L (Lin et al., 2002). Type I RIP have been growing since they are used as components of ‘immunotoxins’, one type of hybrid molecules consisting of a toxic peptide chain linked to an antibody (Frankel et al., 1986). To elaborate the purification methods and investigate the antitumor activity of the recombinant protein of curcin, the fragment encoding the mature protein of curcin was inserted into $E. \text{coli}$ strain M15 and the recombinant strain was induced to express by the optimum inducer (0.5 mM isopropyl- $\beta$ -D-thiogalactopyranoside). The recombinant protein was expressed in the form of the inclusion body and was purified by Ni-NTA affinity chromatography. The protein of interest was incubated with the tumor cells at various concentrations for different time. It was shown that the target protein could inhibit the growth of NCL-H446, SGC-7901, and S180 at a very low concentration (Luo et al., 2006). RIP gene, curcin was cloned from Jatropha curcas seeds (Lin et al., 2003c). At present, it is useful to look for new RIP to identify those with the highest antitumor activity, to select the most suitable ones for tumor therapy and to overcome the immune response that follows clinically oriented administration of RIP conjugates.

Several studies with animals have shown that the Jatropha seeds are toxic in nature (Adam et al., 1974; Ahmed et al., 1979b; EI Badwi et al., 1995; Liberalino et al., 1988). According to the WSU study trials with Jatropha seed-cake have concluded that its properties compare favorably with those of other organic fertilizers with regard to nitrogen, phosphorus and potassium. However, there are issues needing to be addressed with respect to storage, the formation of organic acids, the slow degradation of lignin (shells) and the possible need for treatment with pesticides that might result from lack of microbial degradation. The improper storage of the seed cake
might also result in the production of toxic aflatoxins (Peter, 2002). However, recent reports have shown that lectin is not the major toxic principle in Jatropha curcas meal (Aderibigbe et al. 1997; Aregheore et al., 1998). The meal of Jatropha curcas has a high activity of trypsin inhibitor and lectin but these can be reduced by heat treatment (Aderibigbe et al., 1997). In addition, high concentration of the antimetabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in J. curcas meal (Makkar et al., 1997). The high concentration of phorbolesters present in Jatropha seed has been identified as the main toxic agent responsible for Jatropha curcas toxicity (Adolf et al., 1984; Makkar & Becker., 1997a). It is not possible to destroy phorbolesters by heat treatment because they are heat stable and can withstand roasting temperature as high as 160 °C for 30 min. however; it is possible to reduce its concentration in the meal by chemical treatments (Makkar & Becker., 1997b).

Main objective is to compile a monograph for J. curcas to validate processes for the detoxification, and give criteria for phytochemical and proteomic characterization of the curcin. Based on these results a main objective is to detoxify the Jatropha curcas cake, so that after detoxification it can be used as animal feed.

The broad objectives of the present study are:

- **Extraction and purification of Curcin toxin from Jatropha curcas seeds**
- **Proteomics studies of Curcin**
- **Immunological studies of Curcin**
- **Toxicity studies of Curcin**
- **Toxicity evaluation of Jatropha Curcas cake**
- **HPLC analysis and Toxicity study of ethanol extracts of Jatropha curcas cake**
- **Detoxification of Jatropha curcas cake**
- **Long term feeding trials of Jatropha curcas cake**