Echinococcosis is a zoonotic infection caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family Taeniidae (Platyhelminthes; Cestoda; Cyclophyllidea). The genus *Echinococcus* consists of five species; *E. granulosus*, *E. oligarthus*, *E. multilocularis*, *E. vogeli* and *E. shiquicus* (Thompson et al., 1995; Xiao et al., 2006). The two major species of medical and public health importance are *E. granulosus* and *E. multilocularis*, which cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. The dog tapeworm *E. granulosus* is one of a group of medically important parasitic helminths of the family Taeniidae that infect at least 50 million people globally (Schantz, 1981; Gracia et al., 2005). Currently, up to 3 million people are infected with *E. granulosus* (McManus et al., 2003; Craig et al., 2007), and, in some areas, 10% of the population has detectable hydatid cysts by abdominal ultrasound and chest X-ray (Li et al., 2011; Moro et al., 1999). Worldwide, echinococcosis causes an estimated annual loss of US $194,000,000 or 285,000 disability-adjusted life years (Budke et al., 2006) Human beings are only incidental intermediate hosts of this parasite. Thus, hydatidosis is a global animal and human health problem. It is common in sheep raising countries like India. In India, *E. granulosus* infections have been identified in different intermediate hosts including buffaloes, cattle (Rao et al., 1986), sheep, goats (Abraham et al., 1980), pigs (Prasad, 1981) and camels (Lodha et al., 1982).

In Himachal Pradesh majority of rural population is involved in sheep and cattle grazing. Dogs are considered as essential part of the household and are kept primarily as guard to protect live stock from wolves. Human exposure to *Echinococcus* egg is likely to be high as dogs are tied in per domestic areas, often close to children and accompany families when they move to different pastures. In general human CE is diagnosed and characterized in the late symptomatic stages when significant pathology has already occurred (Altintas et al., 1998). Thus possibility of high prevalence of *Echinococcus* is likely in Himachal Pradesh. Few reports are available on CE from Himachal Pradesh but the systematic epidemiological study has not been conducted in Himachal Pradesh, the apparently endemic area for human CE.

The life cycle of *E. granulosus* involves two mammals, including an intermediate host, usually a domestic or wild ungulate (humans are accidental intermediate hosts) and a canine-definitive host, such as the domestic dog. The larval (metacestode) stage causes hydatidosis (cystic hydatid disease; cystic...
echinococcosis), a chronic cyst-forming disease in the intermediate (human) host. The larval stage of the parasite produces a large number of infective protoscoleces that develop to adult worms after being ingested by the definitive host, or they produce a new parasite mass when liberated inside the intermediate host, causing metastases of the parasite lesions. *E. granulosus* adult tapeworms are found in nature in the intestines of canines; the larval cyst stage is present in the viscera of herbivores including human beings. In cystic echinococcosis (hydatidosis) of humans, well-delineated spherical primary cysts are formed most frequently in the liver (approximately 65% of the cases), but also in the lungs (25%) and other organs such as kidney, spleen, brain, heart, and bone (Schantz et al., 1986). The host is exposed to long-term damage and immune stimulation, as well as the sheer physical consequences of being inhabited by a large foreign body. The most obvious forms of direct damage from the parasite are those resulting from mechanical blockage of internal organs or from the effects of pressure exerted by growing parasite.

During a parasitic infection, host cell products such as cytokines and lymphocytes are released from activated cells. Immuno-pathological reactions ranges from anaphylactic reactions to cell mediated hypersensitivity (Murray et al., 2005). These facts determine all aspects of interest to the disease.

The symptoms of hydatid disease in man are variable and its clinical diagnosis is difficult. Early diagnosis of the disease is important, because radical removal of metacestode tissue by surgery is considered as the most successful treatment. However, diagnosis very often is delayed because cysts develop slowly and little specific symptoms, such as abdominal pain, nausea, jaundice and feeling of exhaustion occurs. Up to 50% of alveolar echinococcosis (AE) and cystic echinococcosis (CE) cases may remain asymptomatic and parasite lesions would incidentally be detected during examinations for other diseases. Current routine diagnosis of human echinococcosis is based on imaging procedures i.e. ultrasound, x-ray, computed tomography and magnetic resonance imaging (Siles-Lucas et al., 2001). Immunodiagnostic techniques such as ELISA and immunoblotting are currently applied to confirm the presence of an *Echinococcus* cyst (Eckert J et al., 2004).

The laboratory diagnosis mainly depends upon the detection of antihydatid antibodies in serum samples. Among the serological techniques, ELISA is sensitive
assay to diagnose CE. Hydatid cyst fluid has been used as a main antigenic source for
the primary immunodiagnosis of human cystic echinococcosis (Ortona et al., 2003).
ELISA using crude hydatid cyst fluid (EgHF) has a high sensitivity (75 - 95%), but its
specificity is often unsatisfactory (Keller et al., 2002). The main problem is its cross-
reactivity with sera from individuals infected by other helminths, mainly *E. multilocularis* and *Taenia solium* (Leggatt et al., 1992). So far there is no standard, highly sensitive and specific test available for the diagnosis of the disease in humans (Li et al., 2003). It was also shown that carbohydrate and lipid content varies between fertile and non-fertile cysts. Thus, there is somehow a lack of standardization in using a native parasite material as main source of antigen due to varying content of its components (Siles-Lucas et al., 1998). Thus source and fertility of cysts seem to be critical for test outcome.

It has been suggested that serodiagnosis of CE may be improved by use of recombinant proteins, synthetic peptides or combinations of well-defined antigens that enhance diagnostic specificity (Zhang et al., 2003). To overcome problems associated with native antigen, several recombinant antigens from both, *E. granulosus* and *E. multilocularis* have been produced and tested, for example recombinant *E. multilocularis* antigen II/3-10, which has a long history of successful application in diagnosis of human AE (Rodrigues et al., 1993; Ferreira and Zaha, 1994; Siles-Lucas et al., 2001). Benefits of synthetic peptides are unlimited availability, stability and reproducibility. However, in some cases, the use of such recombinant components still exhibit low diagnostic sensitivities. Thus, to improve the immunodiagnosis of CE, the characterization and standardization of new antigens is the most important tasks to be undertaken.

Further it is postulated that the strain variation in parasite may influence host specificity, life-cycle patterns, development rate, transmission dynamics, antigenicity, diagnosis and sensitivity to chemotherapeutic agents. Therefore it may have implications for the development and design of vaccines and diagnostic reagents (Craig et al., 2007). Genetic diversity within different species of parasites is a major reason as to why parasites survive despite the ability of their host to mount immune responses, which are effective in eliminating a particular infection. Extensive genetic variation has been reported in *E. granulosus* from different parts of world including India (Sharma et al., 2013). Identification of *E. granulosus* using morphological and
biological features is very difficult and labor-intensive. Therefore, strain identification using molecular techniques is the preferred method today. *E. granulosus* is a complex of species/strains which exhibit diversity in their life cycle patterns and host range (Thompson *et al.*, 2002). So far, 10 genotypes of *E. granulosus* have been identified by molecular genetic analysis using mainly mtDNA sequences (Bowles *et al.*, 1992; Scott *et al.*, 1997; Lavikainen *et al.*, 2003). Genotyping of human CE is useful to assess the parasite transmission patterns for epidemiological purposes and the human susceptibility to a particular genotype of *E. granulosus*.

For the treatment of CE, the most preferred option is radical resection of the parasitic mass. Surgery is accompanied by chemotherapy, and in inoperable cases, chemotherapy is the only option. Albendazole and mebendazole are currently used chemotherapeutic drugs (Hemphill and Walker, 2004; Hemphill *et al.*, 2007). However, treatment failures and/or the occurrence of several adverse effects have been reported from these drugs. The adverse effects of benzimidazoles include neutropaenia, proteinuria, mild hepatotoxicity (transient increase of aminotransferases), gastrointestinal disturbances and transient alopecia. The potential risks of benzimidazoles include embryotoxicity and teratogenicity have been observed in laboratory animals during the early stages of pregnancy (Reuter *et al.*, 2004). Therefore, the search for novel drug candidates for the treatment of the disease has to be pursued. Improved drug treatments with lesser side effects are needed (Reuter *et al.*, 2004; Hemphill and Muller, 2009).

Keeping all these points in view, the present study was planned to determine the prevalence and molecular characterization of *E. granulosus* in Himachal Pradesh. Identification of strain types of *E. granulosus* will have immense impact on planning, execution and implementation of hydatidosis control programme. Our study will also focus on studying a range of diagnostic, drug and vaccine targets that can facilitate the development of new intervention tools for hydatid diagnosis, treatment and control.
1.1 Aim and Objectives:

The aim of the present study was to investigate the prevalence and molecular characterization of Cystic Echinococcosis in Western Himalayan region of Himachal Pradesh followed by identification of immunodominant peptides for vaccine and diagnostic use and screening of amide based compounds for anti-echinococcal activity.

OBJECTIVES
1. To determine the prevalence of Cystic Echinococcosis in Solan district of Himachal Pradesh using sero-diagnostic approach.
2. Molecular characterization of strains by using PCR-sequencing analysis of nd-1 gene and cox-1 region of *E. granulosus*.
3. Identification and molecular docking of promiscuous T-cell and B-cell epitopes targeting vaccine candidates: Eg95, Reticulon-4, LDL-receptor, Tetraspanin and Glutathione-S-transferase proteins of *E. granulosus*.
5. *In silico* study, synthesis, characterization and analysis of anti-echinococcal activity of amide based compounds.