1. INTRODUCTION

Neglected tropical diseases (NTDs) are a group of chronic and debilitating conditions, caused by parasitic, bacterial, and viral infections, that include, among others, Schistosomiasis, Cysticercosis and Hydatoidosis (WHO, 2013). These NTDs are the most prevalent diseases in the poorest populations in the world, keeping an estimated 2.7 billion people at risk and a cause of morbidity in the tropical countries (Hotez *et al*., 2007).

Praziquantel (PZQ) is a pyrazino-isoquinoline drug used in both veterinary and human medicine as the drug of choice against many parasitic diseases caused by cestodes and trematodes. It is widely used in developing countries for the treatment of Schistosomiasis and Neurocysticercosis (Sotelo *et al*., 1984). Moreover, PZQ has become the cornerstone for Hydatid control campaigns worldwide (Urrea-París *et al*., 2001). For its efficacy, safety and comparative cost-effectiveness, PZQ is included in the World Health Organization model list of essential drugs (WHO, 2013; Lindenberg *et al*., 2004).

Schistosomiasis, also known as bilharziasis, is a common intravascular trematode infection that is second only to malaria in public health importance in tropical countries. More than 779 million people are at risk and about 207 million actually infected in 74 countries (Steinmann *et al*., 2006). Unlike other infestations, schistosomal infestations can be considered

systemic, as the parasites are localized in organs other than the gut (Martindale, 1993; Chitsulo *et al*., 2000). The current treatment strategy, recommended by WHO, is to control morbidity through preventive chemotherapy with praziquantel (WHO, 2013).

Human cysticercosis is caused by the development of *Taenia solium* cysticerci in human tissues. Cysticerci that develop in the central nervous system cause Neurocysticercosis (NCC). NCC is the most frequent preventable cause of epilepsy in the developing world. More than 80% of the world's 50 million people who are affected by epilepsy live in developing countries, many of which are endemic for *T. solium* infections in people and pigs. The disease affects from 20% to 50% of late-onset epilepsy cases globally and is reported to be a common cause of juvenile epilepsy in certain countries, such as India and South Africa. Praziquantel produces spastic paralysis of the parasite and destroys the scolex. About 60% to 70% of parenchymal cysticerci disappear after a 15-day treatment at doses of 50 mg/kg per day (Nash, 2003; Sotelo *et al*., 1984; Sotelo *et al*., 1985).

Cystic Echinococcosis (CE) is caused by infection with the larval stage (hydatid) of the cestode *Echinococcus granulosus* and is one of the world's major zoonotic infections (WHO 2013; Schantz, 1991). Humans acquire infection by accidental ingestion of *E. granulosus* eggs voided in the feces of infected dogs and the disease is common in parts of the world where there is close contact between the intermediate and definitive hosts, usually sheep

and dogs, respectively (Gottstein and Reichen, 2003). Cystic Echinococcosis or Hydatoidosis is caused by *Echinococcus Granulosus* while Multilocular Echinococcosis is caused by *Echinococcus Multilocularis*. The CE is widely distributed in southern South America, the Mediterranean coast, the southern part of the former USSR, the Middle East, south-western Asia, northern Africa, Australia, New Zealand, Kenya and Uganda. On the Tibetan Plateau *E. Granulosus* cysts have been found in 6.6% of the population. The distribution of *E. multilocularis* is limited to sub arctic regions of Alaska and Canada, central and eastern European countries. In Asia, it is found in the former USSR, Turkey, Iraq, northern India, Japan and central China. Praziquantel is protoscolicidal agent and may act synergistically with albendazole (Morris *et al*., 1986; Taylor and Morris, 1989).

PZQ also has potent cestocidal activity against *Taenia taeniaeformis*, *Mesocestoides vogae, M. corti, Dipylidium caninum*, and *Hymenolepis diminuta* in animals and humans (Thomas and Gonnert, 1977; Gemmell *et al*., 1977). PZQ is highly effective on mature and immature tapeworm species tested in mice, rats, cats, dogs, and sheep (Thomas and Gonnert, 1977).

Although PZQ is a very effective anthelmintic, it exhibits poor oral bioavailability because of its low aqueous solubility, extensive hepatic first-pass metabolism and the short plasma half-life (0.8-1.5 hours) (Bittencourt *et al*., 1990, Caffrey, 2007; Cioli and Pica-Mattoccia, 2003). It also suffers with great pharmacokinetic and therapeutic variability after its oral

administration (Jung *et al*., 1990). These shortcomings necessitate its frequent administration and limit its use for the above parasitic diseases. The common usage of PZQ is up to 75 mg/kg per day for 15–20 days preoperatively for hydatid disease caused by *Echinococcus* spp. (Cobo *et al*., 1998). The dose of PZQ is 50 mg/kg/day for 15 days for the cysticercus stage of *T. solium*, especially in its localization in the brain (NCC) (Bale, 2000). Repeated administrations of high oral doses of PZQ are necessary to overcome first pass metabolism and thereby achieve sufficient concentrations of PZQ at the larval tissue for therapy of cestode infection (Leopold *et al*., 1978). Moreover, frequent administration of high oral doses is not only time-wasting, but also costly and further results into patient incompliance and inconvenience. The high daily doses might produce a slight transient disturbance in general wellbeing, such as tiredness, dizziness, nausea, and hangover feeling (Leopold *et al*., 1978).

The development of resistance to PZQ in certain countries also reported (Caffrey, 2007; Doenhoff and Pica-Mattoccia, 2006). To overcome the above mentioned shortcomings, novel strategies for delivery of PZQ are necessary. In previous studies, researchers have focused either to increase PZQ concentrations in plasma using concomitant administration with cimetidine, grape fruit juice or food (Jung *et al*., 1997; Castro *et al*., 2002; Castro *et al*., 2003; Mandour *et al*., 1990) or to improve the dissolution rate using adjuvants such as cyclodextrins and polyvinylpyrrolidone (El-Arini and

Leuenberger, 1998; El-Arini and Leuenberger, 1996; Becket *et al*., 1999). Liposomes have also been attempted to improve bioavailability as well as the antischistosomal activity of PZQ (Akbarieh *et al*., 1992; Maurao *et al.*, 2005). Some researchers have developed the long-term sustained-release implantable PZQ-containing bar and PZQ-loaded sustained-release PCL implant for veterinary use (Jiao *et al*., 2005; Cheng *et al*., 2010). The strategies currently being investigated are the improvement of oral bioavailability using polymeric and solid lipid nanoparticles (SLN) and to assess the alternative routes for drug administration (Mainardes and Evangelista, 2005; Yang *et al*., 2009; Xie *et al*., 2010).

Nanosponges (NS) represent one of the novel interesting approaches to increase the solubility of the poorly soluble drug. Cyclodextrin based nanosponges are recently developed hyper cross-linked cyclodextrin polymers nanostructured to form three-dimensional networks; they are obtained by reacting cyclodextrin with a cross-linker such as Carbonyldiimidazole, Dimethyl carbonate, Diphenylcarbonate etc (Trotta *et al*., 2003).

NS are solid particles with spherical morphology that have been reported to have a very high solubilizing power for poorly soluble molecules, to protect degradable molecules, to mask unpleasant flavors and to formulate drug delivery systems for various administration routes such as Oral, Parenteral, Topical or Inhalation routes (Trotta *et al*., 2003). For the oral administration,

the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets (Jenny *et al*., 2011). The nanosponges has been used in the formulation of drugs such as Tamoxifen (Jenny *et al*., 2011), Paclitaxel (Torne *et al*., 2010; Ansari *et al*., 2011), Camptothecin (Swaminathan *et al*., 2009; Rosalba *et al*., 2011), Resveratrol (Khalid *et al*., 2011), Itraconazole (Swaminathan *et al*., 2007), Dexamethasone (Lala *et al*., 2011), Curcumin (Darandale and Vavia, 2012), Telmisartan (Rao *et al*, 2012).

Furthermore, nanosponges show a remarkable advantage in comparison with the common nanoparticles: indeed, they can be easily regenerated by different treatments, such as washing with eco-compatible solvents, stripping with moderately inert hot gases, mild heating, or changing pH or ionic strength. The important and innovative aspects of these nanosponges are their lack of toxicity and their ability to be combined in spherical particles on a micrometric scale. The cyclodextrin cross-linker ratio can be varied during their preparation to improve the drug loading and solubility.

Intestinal lymphatic delivery has also emerged as a means for site-specific oral absorption of peptides, proteins, drugs, and vaccines (Rawat *et al*., 2011; Paliwal *et al*., 2009). The unique advantage is to avoid first pass metabolism following peroral drug delivery. Gastrointestinal tract is richly supplied with blood and lymphatic vessels. Since rate of fluid flow in portal blood is about 500 fold higher than that in intestinal lymph, the majority of the dietary

compounds are transported to portal blood (Holm and Hoest, 2004; Manjunath and Venkateshwarlu, 2005). Highly lipophilic compounds such as long chain triglycerides with chain lengths of 14 and above could reach systemic circulation via the intestinal lymphatics (Porter and Charman, 2001; Caliph *et al*., 2000; Holm *et al*., 2001). Several mechanisms, for delivering drugs to or through lymphatics following the peroral drug delivery include paracellullar mechanism (Yoshikawa *et al*., 1981; Muranishi *et al*., 1997), transport through M cells of Peyer's patches (Hussain *et al*., 1997; Clark *et al*., 2001) and transcellular mechanism (Paliwal *et al*., 2009; Holm and Hoest,2004;Porter and Charman,2001; Caliph *et al*.,2000). Among them, transcellular absorption is the most promising mechanism for the absorption of lipid carriers. The strategies such as prodrug synthesis, permeation enhancers, liposomes, microemulsions, polymeric nanoparticles, self-emulsifying drug delivery systems (SEDDS) and solid lipid nanoparticles (SLN) have been explored for the delivery of bioactives to intestinal lymphatics (Paliwal *et al*., 2009, Rawat *et al*., 2011).

Solid lipid nanoparticle (SLN) represents an alternative carrier system to traditional colloidal carriers (emulsions, liposomes and polymeric micro or nano particles) in enhancing the oral bioavailability of poorly soluble drugs. These particulate systems contain solid lipids (that remain in the solid state at room and body temperatures) as matrix material which possesses adhesive properties that make them adhere to the gut wall and release the

drug exactly where it should be absorbed (Muller and Keck , 2004). Lipid matrix of SLN is made from physiologically tolerated lipid components that include: fatty acids, steroids, waxes, mono-, di-, or triglyceride mixtures. To stabilize SLN, a wide variety of biocompatible surfactants have been used which decreases the potential for acute and chronic toxicity (Mehnert and Mader, 2001). They offer the advantages over traditional colloidal systems such as enhanced physical stability, protection of drug degradation in the body, possibility of controlled drug release, low or total absence of toxicity, drug targeting and different possible administration routes (Muhlen *et al*., 1998). Drugs with poor oral bioavailability due to low solubility in GI tract or pre-systemic hepatic metabolism (first-pass effect) can be incorporated into SLN. The lipid core of SLN may mimic chylomicron formation by enterocytes, which dissolve and assimilate lipophilic drug molecules and promote the absorption of water-insoluble drugs into intestinal lymphatics by the transcellular mechanism of lipid absorption, thereby reducing hepatic first pass metabolism by allowing transportation of drug via the intestinal lymphatics, which directly drain into thoracic duct, finally into the venous blood, thus by passing the portal circulation (Porter and Charman, 2001; Paliwal *et al*., 2009). Previous studies demonstrate that SLN as carrier for bioactives through lymphatic regions following oral administration prolong the systemic circulation time and increase the bioavailability of drugs including Methotrexate Repaglinide and Lopinavir etc (Paliwal *et al*., 2009;

Rawat *et al*., 2011 Bargoni *et al*., 1998; Alex *et al*., 2011). In case of schistosomiasis, the lower plasma levels and longer duration of action of praziquantel may be advantageous in reducing side effects and prolonging exposure of the schistosomes to the drug (Mandour *et al*., 1990). Due to their particulate nature and inherent structure, SLN exhibit good potential in the treatment of parasitic infections (Date *et al*., 2007).

However, despite the initial claims regarding their potential for the physical storage stability, so far, SLN have shown instability during storage, owing to the crystallinity and polymorphic behavior of lipids. In general, drug molecules stay in between the fatty acid chains or as amorphous clusters in crystal imperfections within SLN matrix. Entrapment efficiency of the drug in the lipids depends upon the factors such as miscibility, solubility of drug in lipid melt, physical and chemical structure of the lipid matrix and polymorphic state of lipid materials. More complex lipids (triglycerides) form less perfect crystals with many imperfections. These imperfections offer space to accommodate the drug. Polymorphic transitions of the lipid may occur with time due to the crystalline structure of solid lipid. Thus, when lipid transform to low-energetic form, it form a perfect crystalline lattice that allows very small space for the drug molecules. Therefore, expulsion of encapsulated drug molecules may be observed during storage, especially when SLN matrix is composed of a highly purified lipid, which leads to limited drug-loading capacity of SLN (Das *et al*., 2011; Müller *et al*., 2000;

Bunjes *et al*., 1996). Therefore, the entrapment efficiency (EE), physical stability and drug release kinetics of SLN may change with storage time.

To overcome the above mentioned drawbacks the nanostructured lipid carriers (NLC) were introduced. In NLC some part of the solid lipid was replaced with the liquid lipid. The addition of liquid lipid increases the drug solubility and simultaneously allowing more space to accommodate the drug (Jenning *et al*., 2000). In previous studies by our group, binary lipid nanoparticles has been tried by including mixture of two solid lipids (Rawat *et al*., 2011). These binary lipid matrix created deformation in crystal order of lipids and thereby increased drug loading with very low possibility of the drug expulsion.

Based on the above discussion, the proposed research work was aimed to improve the bioavailability and systemic circulation time of PZQ by incorporation in suitable novel drug delivery systems which in turn could enhance its therapeutic efficacy as well as reduce dose and dosage regimen. For this purpose, PZQ was incorporated in two types of nanoparticulate carrier systems such as NS and SLN. The comparative effectiveness of these carriers was evaluated. Out of the duo, NS were principally used to increase the dissolution of the PZQ in the physiological media while SLN were used to increase the intestinal solubilization and targeting the intestinal lymphatic delivery system and thereby avoiding the first pass metabolism.

The first part of the present research work was devoted to prepare and characterize cyclodextrin based nanosponges (NS), which behave as innovative drug carrier. Drug molecules to be complexed with nanosponges should have certain characteristics such as molecular weight between 100 and 400, drug molecule consists of less than five condensed rings, solubility in water is less than 10mg/mL and melting point of the substance is below 250°C. PZQ fulfills all of the above criteria to be successfully loaded in the nanosponges. NS were formulated using different ratios of cross linker (Dimethylcarbonate). The complexation of drug in to the NS was assessed through FTIR, DSC and XRD studies of NS complexes. The formulations were evaluated for their capacity to incorporate PZQ within their structure and enhancement in solubilisation efficiency. The morphological characterization is also carried out using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The *in vitro* release was studied in two different physiological media. The pharmacokinetic behavior of PZQ-NS was evaluated in rats.

In the second part of the present research work, PZQ–loaded SLN were designed and developed for peroral and parenteral administration. SLN were prepared by a hot homogenization followed by ultrasonication method using triglycerides as lipid core materials. Lecithin granular was used as lipophilic emulsifier or co-surfactant and Poloxamer 188 was used as a hydrophilic emulsifier. The effects of various process and formulation parameters such

as homogenization time, ultrasonication time and surfactant/lipid composition on the physicochemical properties of SLN were investigated. SLN were further characterized for their mean particle size and morphology to correlate their structural resemblance to chylomicrons and *in vitro* release in simulated pH conditions encountered en route on oral administration, so as to assess the potential as oral formulation. The stability behavior in terms of size and entrapment efficiency of the formulations with time at various storage conditions and pH conditions were studied to evaluate the effect of the various pH environments of the gastrointestinal tract (GIT) on drug and carrier stability as well as the stability during the harsh procedures of autoclaving. The *in vivo* pharmacokinetic study was conducted to assess the bioavailability of praziquantel SLN after parenteral and oral administration. The intestinal transport of SLN after intra-duodenal administration was also investigated to clarify its lymphatic delivery. Further, with a view to improve the drug loading, praziquantel (PZQ) loaded binary solid lipid nanoparticles (BSLN) were formulated using binary lipid matrix (combination of lipids). BSLN were prepared by hot homogenization followed by ultrasonication method using various ratios of glyceryl mono stearate and triglyceride as lipid core materials. Lecithin granular was used as lipophilic emulsifier or cosurfactant and Poloxamer 188 was used as a hydrophilic emulsifier. The BSLN were also subjected to stability studies, *in vitro* release studies and *in vivo* pharmacokinetic studies. The therapeutic efficacy of the PZQ loaded SLN

was also assessed *in vivo* against experimental (*Hymenolepsis diminuta*) infection in rats. The adult worms live in the small intestine of final hosts, rats and other rodents (Dixon and Arai, 1980). Because of the easy maintenance of the *H. diminuta* cycle in the laboratory for many years, this helminth is often used as a model for other cestodes.