Rationale for present investigation
Transdermal drug delivery is an attractive alternative to conventional techniques for administration of systemic therapeutics. One challenge in designing transdermal drug delivery systems is to overcome the natural transport barrier of the skin. The outermost layer of skin, stratum corneum, is primarily composed of dead corneocytes embedded in lipid layers (Elias, 1983). This brick and mortar like arrangement offers a substantial barrier to small hydrophilic compounds as well as to essentially all high molecular weight drugs. Molecules which are successful in crossing stratum corneum may enter the blood circulation via diffusion (Flynn et al., 1974). The rate of diffusion depends on molecular weight as well as concentration gradient, making it even more difficult to deliver large molecules in a time controlled manner, as macromolecules diffuse slowly and may have limited solubility in aqueous medium. This has limited the number of drugs delivered with passive methods to lipophilic molecules under 500Da (Prausnitz et al., 2004). Therefore, there is a need for methods and devices to deliver hydrophilic and high molecular weight drugs in a controlled and reproducible fashion.

Technologies used by transdermal devices can be divided into passive or active methods based on whether an external source of energy is used for skin permeation enhancement. Passive methods include use of chemical enhancers, emulsions and lipid assemblies as well as biological methods such as peptides (Schreier and Bouwstra, 1994; Karande et al., 2004; Prausnitz et al., 2004). Chemical methods are relatively easy to incorporate into transdermal patches and can be used to deliver varying dosage amounts by changing the application area. However, these methods may have a lag time up to hours and thus cannot be easily adapted for rapid onset or time varying delivery which may be needed for drugs such as insulin. Increasing numbers of academic and industrial researchers are focusing on transdermal devices with active mechanisms for skin permeation. A similar trend is seen in the type of systems that have entered the transdermal market in the last decade, and those under clinical development (Gordon and Peterson, 2003). These active methods of skin permeation enhancement include jet injectors,
iontophoresis, electroporation, ultrasound, microneedles, powder injection, ablation and tape stripping (Arora et al., 2008).

Over the last decade, great progress on this front has been made with the advent of devices which have at least one working parameter in micrometer range and are collectively referred to as micro-scale devices. Such micro-scale devices include liquid jet injectors, solid powder injectors, microneedles and thermal microporation devices.

One long-standing approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters or accelerants) which penetrate into skin to reversibly decrease the barrier resistance. Numerous compounds have been evaluated for penetration enhancing activity, including sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol, or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes. Many potential sites and modes of action have been identified for skin penetration enhancers; the intercellular lipid matrix in which the accelerants may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue by acting as a solvent for the permeant within the membrane. Further potential mechanisms of action, for example with the enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin, or exerting an influence on the thermodynamic activity/solubility of the drug in its vehicle are also feasible. Chemicals offer tremendous potential in overcoming the skin barrier to enhance transport of drug molecules. Chemicals are however limited in their efficacy in disrupting the skin barrier at low concentrations and usually cause skin irritation at high concentrations.

The use of synthetic chemical enhancers for enhancing the percutaneous permeation of drugs is decreasing due to their long term effects and potential systemic toxicity (Lashmar et al., 1989). Hence, herbal enhancers are being widely investigated for percutaneous permeation
enhancement. Hence, the main objective of this study was to evaluate the influence of alkaloids and coumarins on percutaneous permeation of repaglinide and to understand the mechanism(s) responsible for this effect. Compounds with high lipid solubility cause changes in membrane dynamics because of easy partitioning in a lipid matrix. They are capable of entering the hydrophobic region of both lipids and proteins in the membrane core (Goldstein, 1984). Chemico-biological interactions of piperine enhances the bioavailability of various structurally and therapeutically different drugs (Zutshi et al., 1985; Bano et al., 1987, 1991; Annamalai and Manavalan, 1990). Piperine is absorbed very quickly across the intestinal barrier. It is reported to exhibit passive diffusion with nonsaturable absorption kinetics, short absorption clearance and a high permeability co-efficient (Khajuria et al., 1999). It is, therefore, reasonable to presume that since piperine is an apolar molecule, it may modulate membrane dynamics due to its easy partitioning in the hydrophobic core and assist easy permeation of solutes. Piperine modulates permeability characteristics. Fang et al. (1999) studied that the incorporation of nonivamide could increase the in vivo transdermal permeation of sodium nonivamide acetate. The aim of the study was to investigate the effect of capsaicin and nonivamide on the in vitro transdermal permeation of indomethacin. However, there are experiments done both in vitro and in vivo with the parent compound piperine, which indicate that cosmoperine may operate by increasing either of two events: membrane fluidity, and affinity of nutrient/drug to the cell membrane. It should be also considered that cosmoperine, which is alipophylic compound, may increase solubilization of the intracellular lipid moiety in the skin, making it more permeable to the applied nutrient/drug. Early studies have shown that piperine and other active ingredients present in “hot spices” such as capsaicin (from Capsicum annuum) and zingerone (from Zingiber officinalis), bind a specific receptor, named vanilloid receptor subtype 1 (VR1and increase the intestinal permeability.

With regard to the gastrointestinal tract, it has been shown that piperine reduces intestinal motility both in vitro (Takaki et al., 1990) and in vivo (Izzo et
al., 2001) in the mouse small intestine. So piperine or Zingiber officinalis bioavailability-enhancing property may be attributed to the increase in absorption due to the alteration in membrane fluidity and altered conformation of enzymes associated with the absorption.

The investigations carried out by Beckley-Kartey (1997) revealed coumarins to be extremely rapidly and extensively absorbed across skin into the receptor fluid (Beckley-Kartey et al., 1997). This high topical bioavailability of coumarins was to some extent attributed to the physicochemical properties of coumarins. An optimal lipophilicity (log Po/w =1.2) enables it to be readily taken up into the lipid regions of the stratum corneum. At the same time, the sufficient (albeit low) aqueous solubility allows partitioning from the stratum corneum into the relatively aqueous viable epidermis, dermis, and receptor fluid (Beckley-Kartey et al., 1997). The binding properties of coumarin to target cells are enigmatic, possibly due to biological variability. Diffusion of coumarin molecules through the cell is determined by the phospholipid composition of the plasma membrane. Thus, the level of affinity of coumarins for membrane phospholipids constitutes the driving force that allows the entry of coumarins into the cell.

Coumarins are reported to increase the permeation of compounds due to their ability to easily partition in the membrane (due to its apolar nature) and the configuration they assume with neighboring molecules in the membrane, resulting in altered lipid dynamics (Kao et al., 1988; Ford et al., 2001). Modulation of membrane protein function by its lipid environment is a well known phenomenon (Wang et al., 2000). Coumarins contain benzopyrene ring due to which they affect the protein portion of the skin constituents. Benzo-pyrones are reported to reduce high-protein oedemas through lysis of the excess protein (Camenisch et al., 1998). Coumarin-based cyclic prodrugs of RGD (Arg-Gly-Asp) peptidomimetics, which are fibrinogen antagonists have higher membrane interaction potential as estimated by their partitioning between aqueous buffer and an immobilized artificial membrane than the corresponding RGD analogs. Consequently, these cyclic prodrugs exhibited 5–6-fold greater permeability across monolayers of Caco-2 cells (Wang et al.,
In view of these reports and the observed enhancement of RGE permeation across excised rat epidermis, it was felt essential to investigate the influence of these extracts on skin micro constituents and ultrastructure for gaining an insight into the exact mechanism of percutaneous permeation enhancement.

3-nitrocomarin (3-NC), at concentrations inhibiting phospholipase C-y (PLC- y) possesses the ability to enhance TJ permeability due to its ability to hyperphosphorylate ZO-1 protein. Hence, coumarin could be hypothesized to influence the barrier status of skin though its action on ZO-1 protein. The serine-threonine kinase inhibitor staurosporine is reported to attenuate 3-NC-induced hyperphosphorylation of ZO-2 and increase in tight junction permeability across MDCK cell monolayers. These results provide strong support to the hypothesis that hyperphosphorylation of ZO-2 plays a role in increasing the tight junction permeability across epithelial tissues as exemplified by MDCK cell monolayers.

Being natural in origin they are expected to be safer than the synthetic chemicals that are usually employed as permeation enhancers. It is important to note that however, literature review does not reveal the reports of these extracts for percutaneous permeation enhancement by these extracts. In addition, the mechanism for such an effect by any of these extracts has not been reported.

Repaglinide is a lipophilic and antihypertensive drug. It has a short biological half-life and exhibits a very low oral availability due to extensive first pass metabolism. These facts coupled with the requirements of maintaining constant plasma drug concentration above minimum effective concentration make transdermal route an attractive route of delivery for repaglinide. This route so far has not been exploited for repaglinide.

Hence, the present investigation aimed at studying the role of these extracts in influencing the permeation of lipophilic drug RGE through rat epidermis. In addition, biochemical estimations, transepidermal water loss, biophysical attributes and microscopic manifestations of epidermis after
treatment with these extracts was done to understand the mechanism responsible for percutaneous permeation enhancement of RGE.