Introduction
2. INTRODUCTION

Diabetes mellitus is a complex chronic metabolic disorder, characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms and occurs secondary to an absolute lack of the hormone insulin or decrease in insulin sensitivity (Alberti and Zimmet, 1998). The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world (Carter, 2004). The Diabetes Control and Complications Trial (DCCT) Research Group (1993) stated that tight control of blood glucose is an effective strategy in reducing clinical complications of diabetes mellitus significantly. Diabetes, particularly obesity and metabolic syndrome related T2D is a major health problem in the Western world and it is becoming an increasing threat in developing countries as wealth accumulates and lifestyles change (Zimmet et al., 2001; Astrup et al., 2000).

Obesity is one of the most important contributing risk factors to syndrome X and T2D (Bays et al., 2004; Palou, et al., 2000). Obesity is characterized by hyperinsulinemia (insulin resistance), hyperlipidemia, and hyperglycemia all of which ultimately lead to T2D (Kahn and Flier, 2000). T2D may be caused by a reduced sensitivity to insulin signaling and a reduced efficiency of glucose transport, primarily in adipocytes and muscle cells, leading to hyperglycemia and hyperinsulinemia (Kahn and Flier, 2000). Therapeutically and historically, for biomedical, economical, and convenience reasons, antidiabetic drugs have focused on hyperglycemia, while largely ignoring the problem of overweight or obesity. Further, many of the antidiabetic drugs promote weight gain, i.e., adipogenesis, while reducing blood glucose (Moller, 2001). Dyslipidaemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is one of the many modifiable risk factors. In diabetic dyslipidaemia, lipic abnormalities may be the result of unbalanced metabolic states of diabetes (i.e hyperglycaemia and insulin resistance). Improved control of hyperglycaemic moderate diabetes associated dyslipidemia. However, lipid modifying treatment is warranted in many diabetic patients (Chong and Bachenheimer, 2000). The current strategy should be reducing hyperglycemia, hyperlipidemia, and hyperinsulinemia without increasing adiposity or with a reduction of body weight would constitute a much preferred treatment alternative.
Although, several drugs are available from modern medicine, patients often tend to go for herbal medicines for several reasons. Herbal medicine is an important component of traditional systems of medicines, but authentic reports with respect to modern norms of standardization, diagnosis and efficacy are either not available or scanty. The World Health Organization (WHO) has recently recommend such norms to promote traditional/herbal drugs in National Health Care programs because of their easy availability, low cost, safety and the faith of people in such remedies. Scientific investigation of medicinal plants to identify novel bioactive phytochemical with therapeutic potential has come to be the core area of research in pharmaceutical industry today.

In search of compounds that may be beneficial in diabetes and associated conditions like obesity, hyperlipidemia and diabetic cardiomyopathy, we have explored the possibility of polyphenolic compounds like gallotannins to have such potential. Gallotannins are polyphenolic compounds found in legumes, vegetables, fruits and beverages (Okuda et al., 1995). Gallotannins were reported to possess multiple biological activities including anticancer (Okuda et al., 1995), antioxidant (Hagerman et al., 1998) and antimicrobial activities (Cowan, 1999). In recent years gallotannins have been also studied for their antihyperglycemic (Li et al., 2007), lipid lowering (Yunsheng et., 2005; Suzuki et al., 1999) and antioxidant properties (Croft, 1998). Gallotannins from Lagerstroemia speciosa (banaba) are reported to produce antidiabetic activity in in vitro model of diabetic db/db mice and obese ob/ob mice and in invitro studies they also produce glucose transport stimulatory activity in 3T3-L1 adipocyte and adipocyte differentiation inhibitory activity in preadipocytes (Liu et al., 2001). Gallotannin from Punica granatum are reported to enhance cardiac PPAR-γ mRNA expression and restore the down-regulated cardiac glucose transporter (GLUT)-4 in Zucker diabetic fatty rats (Huanga et al., 2005). Gallotannins are reported to reduce blood glucose level not only in experimental animal but also in diabetic patients (Najim et al 2004; Gin et al., 1999). Further, gallotannins produce lipid lowering activity along with glucose lowering activity. In previous reports it has been proved that gallotannins increase peripheral insulin sensitivity in rat adipose tissue by inhibiting lipigenesis (Ong et al., 1995) and produced increased PPAR-γ dependent mRNA expression. Punica granatum flower extract also reported to
produce increase in lipoprotein lipase activity in human THP-1-differentiated macrophage cells in \textit{in vitro} studies (Huanga et al., 2005). In various \textit{in vivo} and \textit{in vitro} animal model, oxygen free radical scavenging activity of different form of gallates, such as gallyl esters, methyl gallate, propyl gallate, gallolectacin, gallotannins, has been documented (Buttemeyer, 2003; Ajay 2003; Calvin and Mattill et al., 1942). Thus, from all these previous reports we can speculate that gallotannins may be responsible for antidiabetic, antihyperlipidemic and antioxidant activities in various medicinal plants and that by virtue of these properties gallotannins may be beneficial in diabetes mellitus.

\textit{E. officinalis} Gaertn. (Family: Euphorbiaceae) commonly known as “Amla” or the Indian gooseberry, has been reported to contain constituents with variable biological activities (Ganju et al., 2003). \textit{E. officinalis} have been shown to possess antioxidant (Bhattacharya et al., 1999; Scartezzini et al., 2006; Anilakumar et al., 2004; Khopde et al., 2001), adaptogenic (Rege et al., 1999), hepatoprotective (Jeena et al., 1999; Achiya et al., 2004), antitumour (Jose et al., 2001), atherosclerosis inhibiting (Thakur et al., 1988), immunomodulatory (Ganju et al., 2003), gastroprotective (Al-Rehaily et al., 2002), hypolipidemid (Ritu et al., 1996), cyto-protective and immunomodulating activity (Sai Ram et al., 2002). The fruits of Amla are used in many medicinal preparations of Ayurvedic and Unani systems of medicine (Kritikar and Basu, 1933). According to the two main classic texts on Ayurved, \textit{Charak Samhita} and \textit{Sushrut Samhita}, Amalaki is regarded as “the best among rejuvenative herbs”.

Phytochemical investigations of fruits of \textit{E. officinalis} show that it is having high amount of polyphenol content like low and high molecular weight gallotannins such as gallic acid, ethyl gallate, digallic acid, ellagic acid, phyllembic acid, chebulinic acid, L-malic acid 2-O-gallate, mucic acid 2-O-gallate, chebulagic acid, putrajivain A, elacocarpusin, mucic acid,1-O-galloyl-β-D-glucose, mucic acid 6-methyl ester 2-O-gallate, mucic acid 1,4- lactone 2-O-gallate, mucic acid 1-methyl ester 2-O-gallate, mucic acid 2-O-gallate, mucic acid 1,4-lactone 6-methyl ester 2-O- galate, mucic acid 1,4-lactone 3- O-gallate, mucic acid 1,4-lactone 3,5-di-O-gallate, emblicanin A and B, punigluconin, pedunculagin, methyl gallate, corilagin, furosin and geraniin. (Ying et al., 2001; Kumaran and Karunakaran, 2006; Anila and Vijayalakshmi, 2003; Ghosal et al., 1996; Sukh, 2006). As mentioned above gallotannins from various plant
sources have been reported to possess antidiabetic, antihyperlipidemic and antioxidant activities. Thus, it is possible that gallotannins present in fruit juice of *E. officinalis* might be having antidiabetic, antihyperlipidemic and antioxidant activities.

Many polyherbal formulations like Trifala, Cogent db, Diasulin contains *E. officinalis* as one of the ingredients have been shown to produce antidiabetic, antioxidant and antihyperlipidemic activity in various animal models (Naik et al., 2006; Saravanan and Pari, 2005; Pari and Saravanan, 2002). It has been reported that extracts of *E. officinalis* reduces the blood sugar levels in alloxan induced diabetic rats (Sabu and Kuttan, 2002; Tripathi et al., 1979). The fruit juice of *E. officinalis* is reported to produce hypolipidemic activity in cholesterol-fed rabbits (Ritu et al., 1996). We have also carried out a preliminary clinical study on diabetic patients in which fresh juice of *E. officinalis* was found to produce significant antidiabetic, antihyperlipidemic and antioxidant activities. No systematic study reported to has been reported to our knowledge for antidiabetic activity of fruit juice of *E. officinalis*.

Gallotannins are hydrolysable tannins which may get hydrolyzed into free form of gallic acid in gastrointestinal tract. Gallic acid is the biological marker compound present in the form of esters in fruit juice of *E. officinalis*. It is reasonable to speculate gallotannin present in *E. officinalis* are hydrolyzed to gallic acid which in turn produces antidiabetic, antihyperlipidemic and antioxidant effect and prevent the development of cardiomyopathy in STZ-diabetic rats.

We made an attempt to verify this hypothesis by first carrying out *in vitro* hydrolysis study of fruit juice in simulated gastric juice and intestinal fluid and the attempt was then made to find out concentrations of gallotannins in fruit juice of *E. officinalis* and its fractions.

We have carried out activity guided phytopharmacological analysis of fruit juice of *E. officinalis* with special reference to gallic acid and studied the effect of fruit juice of *E. officinalis*, its fractions and gallic acid per se in STZ-induced type 1 and type 2 diabetic rats.
The occurrence of hyperglycemia, hyperlipidemia and oxidative stress in diabetes has been extensively documented, and is implicated in the pathogenesis of various cardiovascular complications including cardiomyopathy (Baynes 1991; Cai and Kang 2003; Dhalla et al., 1985; Tomlison et al., 1992; Fang et al., 2004). Thus, we extended our study to investigate the cardioprotective effect of fruit juice and fractions of *E. officinalis* and gallic acid on diabetes-induced myocardial dysfunction in rats.
OBJECTIVES OF THE STUDY

1. To perform activity guided fractionation of fruit juice of *E. officinalis* with special reference to diabetes.
2. Phytochemical standardization of fruit juice of *E. officinalis* and various fractions by HPTLC analysis.
3. To study the fruit juice of *E. officinalis*, fractions and active component in conditions of Type 1 diabetes.
4. To study the fruit juice of *E. officinalis*, fractions and active component in conditions of Type 2 diabetes and obesity.
5. To study the fruit juice of *E. officinalis*, fractions and active component in conditions of diabetes associated conditions like diabetic cardiomyopathy.
6. To carry out *in vitro* hydrolysis study of fruit juice of *E. officinalis*. 