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Antidiabetic effect of some commonly used medicinal plants from Pakistan

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ABSTRACT

The aim of the present study was to investigate the in vitro and in vivo antidiabetic effect of *Elaeagnus umbellata, Phyllanthus emblica* and *Cannabis sativa*. The in vitro antidiabetic activity was studied by the inhibition of alpha glucosidase enzyme. The in vivo antidiabetic activity was studied in albino mice. The diabetes was induced in mice by single interperitoneal injection of alloxan (150 mg/kg). The hot water extracts were fed to the mice orally (250 mg/kg) for seven days. The order of alpha glucosidase inhibitory effect was *P. emblica* > *E. umbellata* > *C. sativa*. The in vivo antidiabetic activity of extracts was further confirmed in alloxan induced diabetic mice. The Normal mice showed glucose level of 110±5 mg/dl. On treatment with single dose of alloxan (150 mg/kg) the sugar level was elevated to 250±10 mg/dl. The treatment with aqueous extract of *E. umbellata* fruit for seven days reduced the glucose level to 220±10 mg/dl. On the other hand treatment with aqueous extracts of *P. emblica* and *C. sativa* reduced the glucose level to 170±11 mg/dl and 185±12 mg/dl respectively. On the basis of these results it is concluded that studied plants have potential to decrease the glucose level by inhibiting the alpha glucosidase enzyme and potentiating of insulin release from pancreas. This will leads to the development of alternative drugs and therapeutic strategies.

KEYWORDS: *E. umbellata, P. emblica, C. sativa,* alpha glucosidase activity, alloxan, diabetes.

INTRODUCTION

Diabetes mellitus is the disorder with severe micro and macro complication that results in significant deaths. It is main cause of death in the world. There are limited effective therapies to cure diabetes. The use of insulin and other antidiabetic agents cause unpleasant side effects, thus

there is need to find safe natural products to treat diabetes. Long term complications such as organs failure are the result of chronic hyperglycemia of diabetes (Olatunde *et al.* 2014). DM also represented by lipidaemia and oxidative stress (Khalid *et al.* 2014). Although many traditional medicinal plants are effective in decreasing blood sugar, most of these are not practically utilized in severe diabetes. The medicinal plants of Azad Kashmir are rich source of antioxidants and phytochemicals (Sabir et al., 2017; Khurshid et al., 2018; Khaliq et al., 2015; Sabir et al., 2015; Sabir and Rocha, 2008).

Phyllanthus emblica L. (emblica) is a medicinal plant which is widely distributed in Asian countries (Verma and Gupta, 2004). The fruit has special taste which is liked by the consumers. The fruit is rich in ascorbic acid and shows superoxide dismutase activity (Verma and Gupta, 2004). Gallic acid, ellagic acid, chebulinic acid, quercetin (Habib *et al.* 2007), kaempferol-3-*O*-α-L-(6″-methyl) rhamnopyranoside and kaempferol-3-*O*-α-L-(6″-ethyl)-rhamnopyranoside (Zhang *et al.* 2005) are present in the fruit of *P. emblica.* The fruit shows hypolipidemic (Anila and Vijayalakshmi, 2004; Jacob *et al.* 1998) and hypoglycemic activities (Abesundara *et al.* 2004) and is used in different hepatoprotective drugs (Antarkar *et al.* 1991; Panda and Kar, 2003).

Elaeagnus umbellata Thunb (autumn olive) belongs to Elaeagnacea family that is native to Southern Europe and Central Asia (Dirr, 1998). This shrub is found in the wild at 1,300-1,800 m above sea level in Azad, Kashmir, Pakistan (Ahmad *et al.* 2006). The fruit is fleshy, sub-globose to broadly ellipsoid, and 6 - 8 mm long. The fruits are light green in mid summer and becomes red in the autumn (Dirr, 1998). The fruit berry is rich in vitamins and contains essential fatty acids (Eckardt and Sather, 1987) and minerals (Matthews, 1994).

Cannabis sativa is used in herbal medicine to treat arthritis or joint pain (Bott and Bishop, 2008). *C. sativa* contains tetrahydrocannabinol (THC), the cannabidiol of *C. sativa* may act as an antipsychotic drug (Turner *et al.* 1980). Terpenes have been detected in essential oil from flowers, leaves, and roots of the plant (Hazekamp and Fischedick, 2012). The terpenes give characteristic flavor to different varieties of cannabis.

Keeping in view the high medicinal uses of these plants, the antidiabetic activities need to be studied in detail. Hence the aim of this study was to evaluate the in vitro and in vivo antidiabetic activities of these plants for their possible use in diabetes.

MATERIALS AND METHODS

The fruits of *E. umbellata* and *P. emblica* while, leaves of *C. sativa* were locally collected and identified by a taxonomist. Finely grounded plant material (25g) was soaked for 15 minutes in boiling water (500 ml), after boiling the extract was cooled and filtered with the help of whatman filter paper No.1. The filtrate was concentrated by rotary evaporator (45^0C) producing 3.5 g (14% w/w) extract.

The institutional and national guidelines for the care and use of laboratory animals was followed. Animal's procedures were approved by ethical council of University of Poonch, Rawalakot (UPR 101). BALB/c mice (20–25 g) were housed in in separate cages where food and water was provided.

Glucosidase inhibitory activity was assessed by the methods described by Sancheti et al. (2011).

The in vivo antidiabetic activity was assessed in BALB/c mice using standard protocols. The 15 mice in group of three comprising five mice in each group. Group 1(control) received distilled water only. Group II (diabetic control) received alloxan 150 mg/kg interperitoneally. Blood glucose levels were monitored in diabetic control group after 72 hours and the mice with glucose level above 200 mg/dl were considered diabetic. Group III (diabetic plant group) received hot water extract at a dose of 250 mg/kg dose orally for 7 days. Blood glucose was estimated with the help of a digital glucometer.

The results were expressed as means± SD. The obtained data was analyzed by one way ANOVA and different group means were compared by Duncan's multiple range test (DMRT) wherever necessary; $p<0.05$ was considered significant in all cases. Statistica was used as software package.

RESULTS

The *Elaeagnus umbellata*, *Phyllanthus emblica* and *Cannabis sativa* showed significant antiglucosidase activity (Fig. 1). The inhibition of alpha glucosidase activity was observed at a concentration range of 100-200 µg/ml and increased with increasing concentration of extract ($P < 0.05$) which indicates that extract possess *in vitro* antidiabetic activity. The order of alpha glucosidase inhibitory effect was *P. emblica* > *E. umbellata* > *C. sativa*.

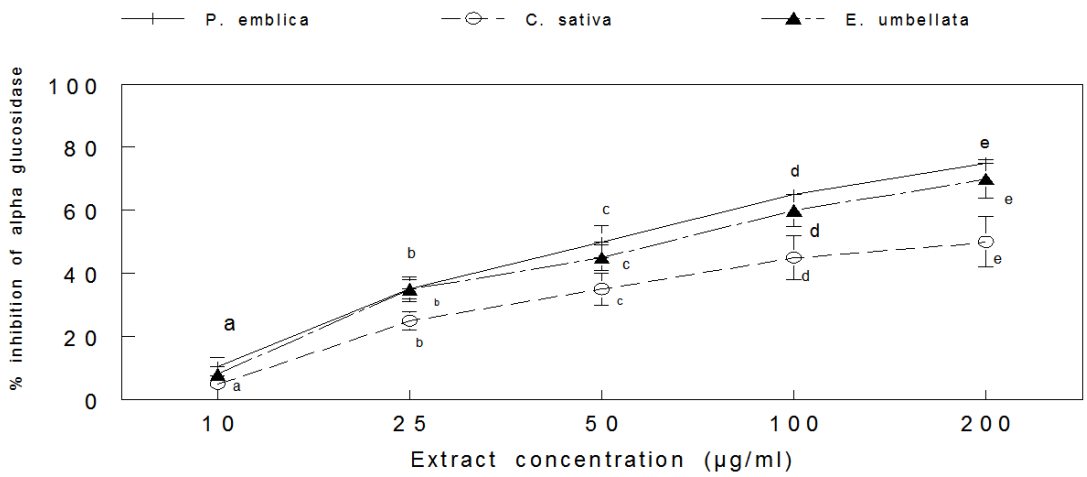

Fig. 1. Glucosidase inhibitory activity by aqueous extract of *E. umbellata* and *P. emblica* and *C. sativa.* Values are means±SD (n=3). Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

The *in vivo* antidiabetic activity of berry extract was further confirmed in alloxan induced diabetic mice (Fig. 2). The Normal mice showed glucose level of 110±5 mg/dl. On treatment

with single dose of alloxan (150 mg/kg) the sugar level was elevated to 250±10 mg/dl. The treatment with aqueous extract of *E. umbellata* fruit for seven days significantly reduced ($P < 0.05$) the glucose level to 220±10 mg/dl. On the other hand treatment with aqueous extracts of *P. emblica* and *C. sativa* significantly reduced ($P < 0.05$) the glucose level to 170±11 mg/dl and 185±12 mg/dl respectively. This shows the antidiabetic effect of these plants in albino mice. *P. emblica* showed the highest percentage in decreasing the sugar level.

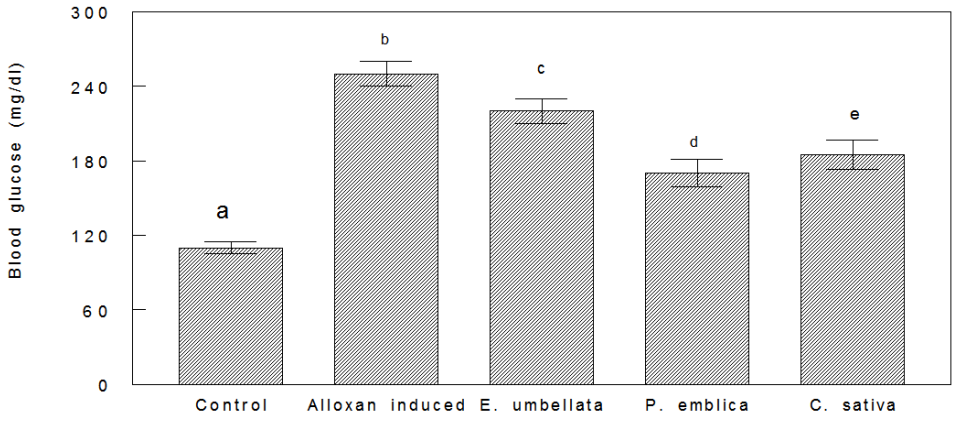

Fig. 2. Antidiabetic activity of *E. umbellate, P. emblica* and *C. sativa* in albino mice. Values are means±SD (n=3). Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

DISCUSSION

Diabetes is one the leading disease effecting 171 million people and most of the patients suffer from type II diabetes (Gershell, 2005). As type 2 diabetes mellitus is increasing day by day and accounts for 9 % of deaths, there is urgent need to find out new pontential therapeutic agents. The treatment for type 2 diabetes mellitus has been improved to some extent during the last decade however; drug resistance is still a big concern. There is need to maintain the blood glucose level and reduce its production to small intestine. Carbohydrate rich diet on consumption increases the absorption of carbohydrate from human intestine due to action of α-glucosidase enzyme which breaks disaccharides into absorbable monosaccharides. The inhibitors of α-glucosidase inhibits the digestion of disaccharides and enable overall smooth glucose profile (Casirola and Ferraris, 2006). Thus, natural products have great diversity in their structure and are potential inhibitors. The alpha glucosidase inhibitory effect of studied plants justifies their popular use in diabetes.

The alloxan-induced diabetic mice had a double elevation in blood glucose levels (110 mg/dl to 250 mg/dl) relative to the normal control mice. There are many studies which show that administration of alloxan a diabetogenic agent induces Type I diabetes in experimental animals (Etuk, 2010; Viana *et al.* 2004). Alloxan monohydrate induces diabetes by selective necrosis of pancreatic beta-cells of Langerhans. This therefore results in hyperglycemia by effecting

endogenous insulin synthesis (Iranloye *et al.* 2005). The toxic alloxan by generation of free radicals, through inhibition of glucokinase enzyme and oxidation of essential suphydryl group induced its toxicities on pancreatic beta cells (Szkudelski, 2001). The underlying mechanism of action involves the selective uptake of the compound due to its similarity to glucose as well as uptake mechanism of the pancreatic beta-cells (Lenzen, 2008). The probable mechanism for the hypoglycemic effect of the aqueous extracts of plants could be linked to potentiation of insulin release from pancreatic beta cells of islet or by elevating the peripheral glucose uptake and utilization (Bedoya *et al.* 1996). Literature showed that saponins and flavonoids are good antidiabetic metabolites (Sharma *et al*. 2010). Our studies are in line with the previous studies (Mehta *et al.* 2009) where *P. emblica* showed antidiabetic activity in animal models. Similarly *C. sativa* have also shown antidiabetic activities against streptozotozocin induced diabetic rats (Levendal and Frost, 2006).

On the basis of these results it is concluded that studied plants have potential to decrease the glucose level by inhibiting the alpha glucosidase enzyme and potentiation of insulin release from pancrease. This will leads to the development of alternative drugs and therapeutics strategies. However, more detailed in vivo assays are required to know the exact mechanisms involved in lowering the glucose level.

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Conflict of Interest statement: Authors declare no conflict of interest involved in the study.

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