Antihyperglycemic effect of *Persea duthiei*on blood glucose levels and body weight in alloxan induced diabetic rabbits

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Abstract: The present study was designed to investigate the antihyperglycemic effect of *Persea duthiei*on blood glucose concentration and body weight in alloxan induced diabetic hyperglycemic rabbits. The results illustrated significant antihyperglycemic activity of crude extract with 17.44% and 28.02% amelioration at 25 and 50mg/kg p.o. respectively after 24th day of drug treatment; equally supported by body weight recovery. Upon fractionation, most dominant antihyperglycemic effect was displayed by aqueous fraction with 22.12% and 34.43% effect followed by ethyl acetate fraction with 24.32% and 32.05% effect at 25 and 50mg/kg p.o. respectively after 24th day of drug treatment. The effect on blood glucose was also reflected on body weight of animals. In conclusion, our study documented marked antihyperglycemic activity of extract/fractions of *P. duthiei*.

Keywords: *Persea duthiei*, extract/fractions, antihyperglycemic activity.

INTRODUCTION

Persea duthiei commonly known as Gul-e-namair is widely distributed around Nainital, Western Himalayas in Pakistan and India to Burma and Skim. This evergreen, small or medium-sized tree grow up to 13cm mostly at altitude of 2500m. The leaves of the plant are united and glabrous, while root stocks are sour, bitter, pungent (Polunin and Stainton, 1984).Several therapeutic uses of the plant are documented such as anti-inflammatory, to relive asthma attack, bronchitis, painful conditions, antiemetic and in blood disease (Padalia *et al*., 2009). It has been showed broad spectrum antifungal and antifungal activity (Ahmad *et al*., 2012).The hypoglycemic effect of other species of genus *Persea* has been reported (Antia *et al*., 2005, Lima *et al*., 2012).

Phytochemically, aporphine alkaloids have been isolated from the root of *P. duthiei* (Hussain *et al*., 1980). Padalia *et al* (2009) has already explored the chemical composition of essential of different parts of the plant. However, other species showed the presence of various phytochemicals such as triterpene glycosides, coumarins, saponins, alkaloids, tannins, reducing sugars, flavonoids, neolignans, glucosylatedabscisic acid derivates (Tsai *et al*., 1998, del Refugio Ramos *et al*., 2004, Lima *et al*., 2012).

The goal of present study was to investigate the anti-hyperglycemic effect of the crude extract and various organic fractions of *P. duthiei* in alloxan induced diabetic rabbits and effect on body weight of test animals.

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MATERIALSAND METHODS

Plant materials

Fresh plants of *Persea duthiei* were purchased from local market in 2013. After collection, plant taxonomist at department of Plant Sciences, KUST, Pakistan determined the taxonomic identities of the desired plants.

Extract preparation

The air-dried and coarsely powdered plant (2.7kg) was extracted three times with methanol. The methanol extracts were evaporated under reduced pressure to give a dark-greenish residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform and ethyl acetate to obtain *n*-hexane soluble, chloroform-soluble, ethyl acetate-soluble and aqueous fractions, respectively. The crude plant extracts and subsequent solvents soluble fractions were then dissolved in distilled water individually and stored in refrigerator at 4°C for future use.

Experimental animals

Healthy rabbits of 1-2 years age (1.2kg-1.5kg weight) purchased from local market, divided into groups; each group contains four rabbits and was assigned to two treatments in a completely randomized design at start of study. Fresh green fodder and tap water were provided to them daily and were placed at room temperature (22-24^{o}C) during the experiment. These animals were approved for experimental purposes and studies by the Ethical Committee of Kohat Institute of Medical Sciences. The blood sugar level of each rabbit was thoroughly recorded with the help of glucometer before the start of the experiment (Abdel-Sattar *et al*., 2011, Sultan *et al*., 2015).

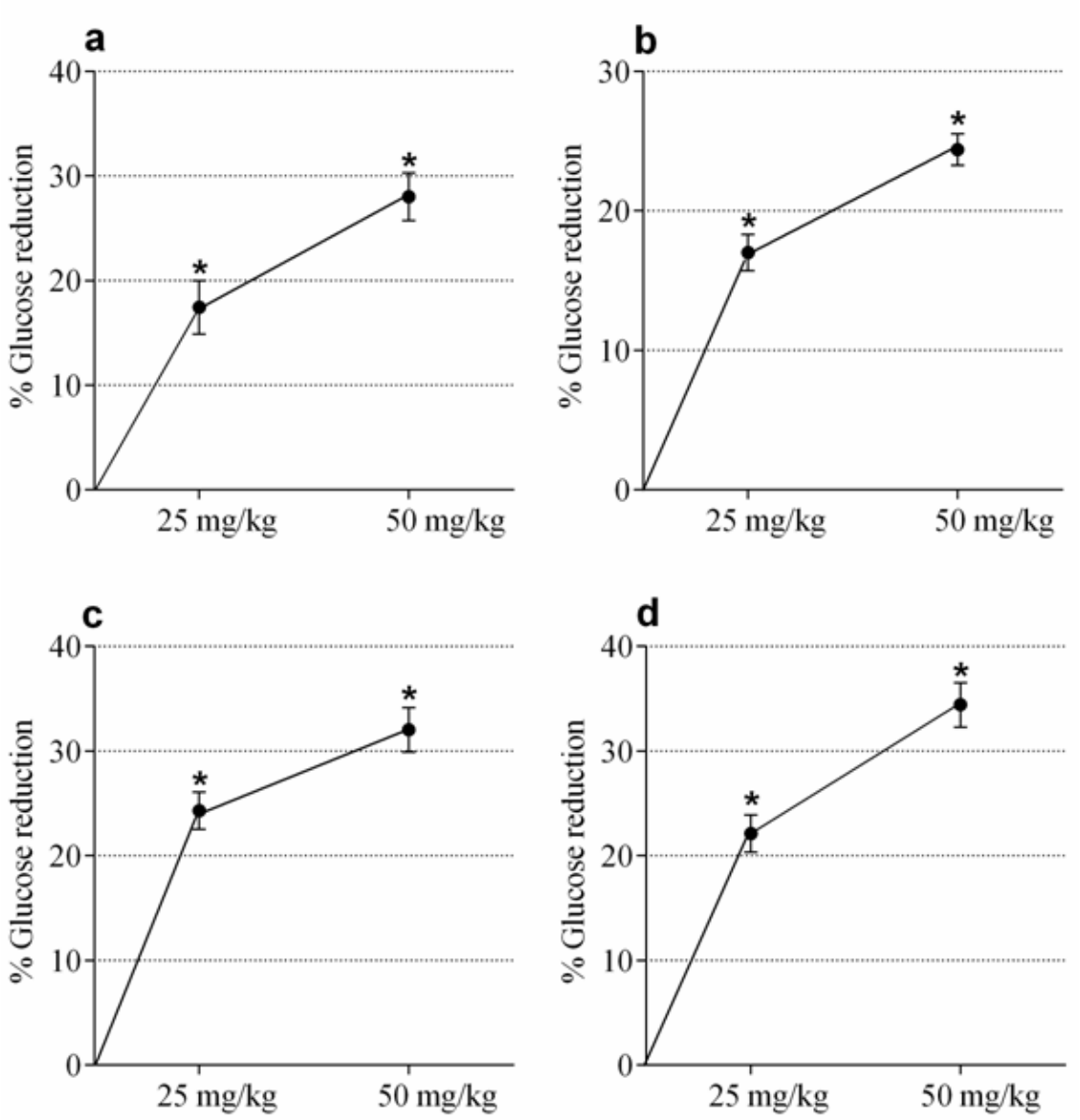

Fig. 1: Effect (%) of *Persea duthiei* [a] crude extract [b] *n*-chloroform [c] ethyl acetate and [d] aqueous fraction son blood glucose in alloxan induced diabetic rabbit. Values are mean ± SEM of four different rabbits. Statistical significance was considered at **P*<0.05 level.

Group a: Normal control (saline).
Group b: Alloxan treated control (150mg/kg.i.v ear vein).
Groups c & d: Alloxan (150mg/kg.ip) + Crude Extract (25mg/kg, p.o; 50mg/kg, p.o),
Group e & f: Alloxan (150mg/kg.ip) + *n*-hexane fraction, (25mg/kg, p.o; 50mg/kg, p.o)
Group g & h: Alloxan (150mg/kg.ip) +Chloroform fraction, (25mg/kg, p.o; 50mg/kg, p.o)
Group I & J: Alloxan (150mg/kg.ip) +ethyl acetate fraction (25mg/kg, p.o; 50mg/kg, p.o)
Group k & l: Alloxan (150mg/kg.ip) +aqueous fraction (25mg/kg, p.o; 50mg/kg, p.o)
Group VII-VIII: Alloxan (150mg/kg.ip) +Standard drug, Glibenclamide (5mg/kg, p.o)

Induction of diabetes

These rabbits were made diabetic by means of alloxan monohydrate. It was administered by i. v ear vein at the dose rate of 150mg/kg body weight daily for one week. This drug inhibits the insulin production, due to which rabbits become artificially diabetic(Ahmed *et al*., 2010). The rabbits with a blood glucose level of 140 mg/dl and above were considered as diabetic. After giving the extracts, their blood was collected from the vein of ear and blood sugar was measured. At day 10, 17 and 24 their blood glucose level were compared with the negative control and effect (%) on total weight was determine as

$$\text{Effect (\%) on body weight} = \frac{\text{After treatment - Before treatment}}{\text{Before treatment}} \times 100$$

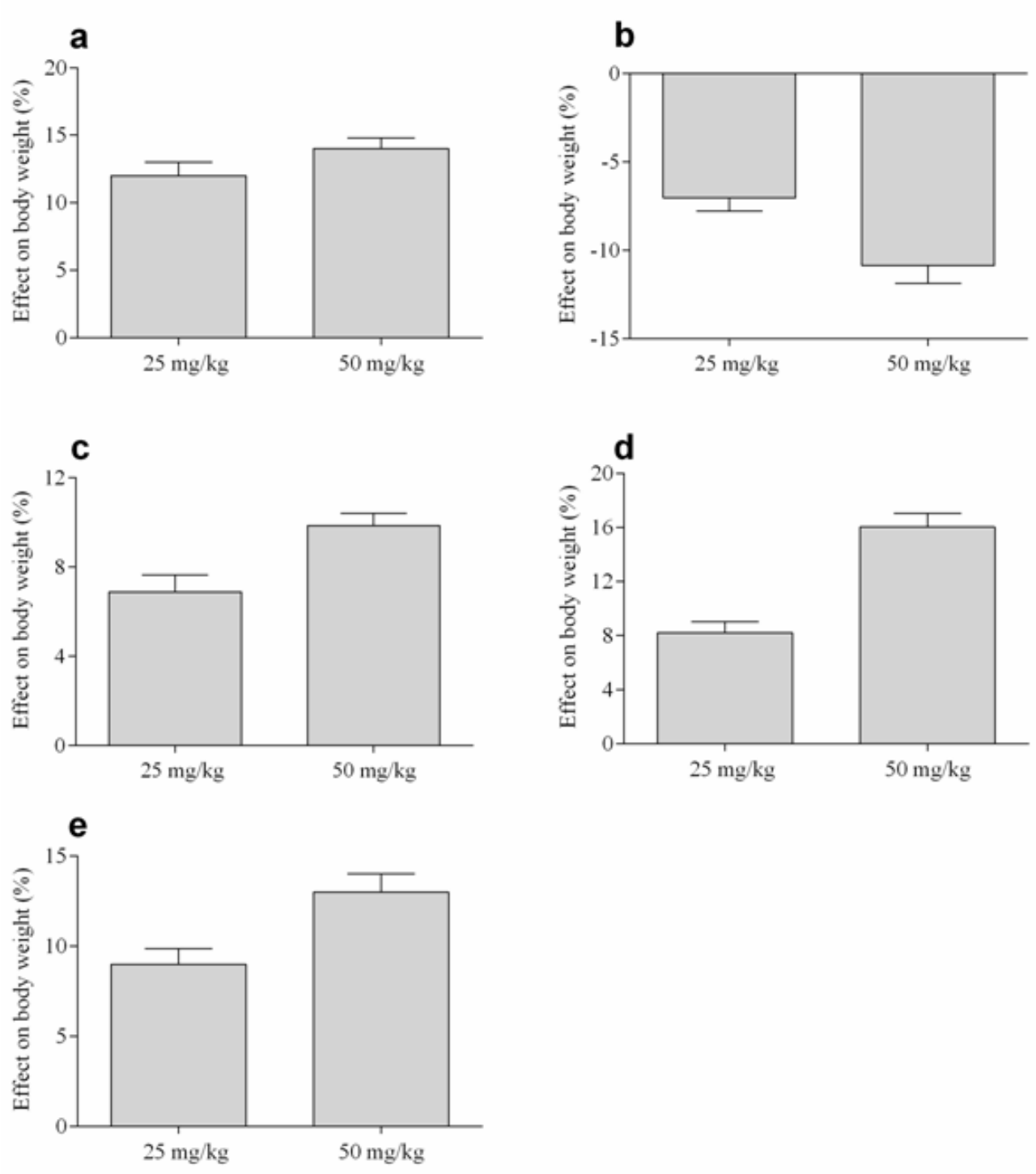

Fig. 2: Effect (%) of *Persea duthiei* [a] crude extract [b] *n*-hexane [c] *n*-chloroform [d] ethyl acetate and [e] aqueous fraction son body weight in alloxane induced diabetic rabbit. Values are mean ± SEM of four different rabbits at the end of treatment.

STATISTICAL ANALYSIS

All values are presented as the mean ±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Dunnet's t-test. Differences between groups were considered significant at $P<0.05$.The statistical analysis was carried out on Graph Pad PRISM 6, San Diego, CA, USA).

RESULTS

Effect of crude extract of P. duthieion blood glucose and body weight

The effect of crude extract of *P. duthiei*on blood glucose in alloxan induced diabetic rabbits is illustrated in table 1. It significantly ameliorated the hyperglycemic effect in a dose dependent manner. The maximum anti-hyperglycemic effect was 17.44% and 28.02%at 25 and 50mg/kg p.o. respectively (fig. 1a) after 24th day of pretreatment. However, the body weight recovery in diabetic rabbits was 11.99% and 14.02% respectively at test doses (fig. 2a).

Effect of hexane fraction of P. duthieion blood glucose and body weight

The result of hexane fraction of *P. duthieion* blood glucose in alloxan induced diabetic rabbits is presented in table 1. It had insignificant effect on blood glucose, which was also reflected on its negative impact on body weight (fig. 2b).

Effect of chloroform fraction of P. duthieion blood glucose and body weight

The chloroform fraction of *P. duthiei* showed significant attenuation of hyperglycemic effect in alloxan induced diabetic rabbits (table 1). It caused 17.01% and 24.39% reduction in blood glucose concentration at 25 and 50mg/kg p.o. respectively (fig. 1b) after 24th day of pretreatment. The body weight recovery in diabetic rabbits was 6.89% and 9.97% respectively at test doses after 24th day of administration (fig. 2c).

Effect of ethyl acetate fraction of P. duthieion blood glucose and body weight

As shown in table 1, the ethyl acetate fraction of *P. duthiei* demon strated marked antihyperglycemic effect on blood glucose. Pretreatment of the fraction provoked 24.32% and 32.05% reduction in blood glucose at 25 and 50 mg/kg p.o. respectively (fig. 1c) after 24th day. Similarly, it produced 8.23% and 16.05%body weight recovery at test doses after 24th day of administration (fig. 2d).

Effect of aqueous fraction of P. duthieion blood glucose and body weight

The result of aqueous fraction of *P. duthiei*on blood glucose in alloxan induced diabetic rabbits was most dominant as presented in table 1. When checked after 24th day of pretreatment, it caused 22.12% and 34.43% reduction in blood glucose concentration at 25 and 50 mg/kg p.o. respectively (fig. 1d). The effect was supported in recovery of body weight with 10.89% and 15.36% respectively at test doses after 24th day of administration (fig. 2e).

DISCUSSION

Hyperglycemia or diabetes mellitus is caused by an inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. The long lasting hyperglycemia causes long-term damages and failure of numerous organs i.e. heart, kidneys, eyes, nerves and blood vessels (El-Missiry and El Gindy, 2000, Chenlin *et al*., 2014). Diabetic patients face various vascular complications in hyperglycemia such as coronary heart disease, atherosclerosis, diabetic nephropathy and neuropathy (Jayakar *et al*., 2004, Abdel-Sattar *et al*., 2013). Such a deficiency results in increased concentration of glucose in the blood, which in turn damage many of the body systems in particular the blood vessels and nerves (Ukwe and Ubaka, 2011, Chenlin *et al*., 2014). Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn lead to secondary complications effecting eyes, kidneys, nerves and arteries. A part from currently available therapeutic options many herbal medicines have been recommended for the treatment of diabetes. Medicinal plants have the advantage of having no side-effects (Ahmad *et al*., 2009, Patel *et al*., 2012).

Several therapies are used in clinical practice for the management of diabetes includes insulin for type 1 (insulin dependent diabetes mellitus) and various oral anti-diabetic agents for type 2 diabetes (non-insulin dependent diabetes mellitus) such as sulfonyl ureas, thiazolidine-diones, α-glucosidase inhibitors etc. These drugs are used as mono-therapy or in combination to achieve better glycemic control (Marles and Farnsworth, 1995, Nabi *et al*., 2013, Chenlin *et al*., 2014). However, they are suffering from multiple problems in terms of patient compliance; the synthetic drugs are not effective controlling hyperglycemia in the majority of patients (Ahmed *et al*., 2010, Nabi *et al*., 2013). Hence the antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects.

Plant kingdom has an incredible therapeutic history in the effective management of different diseases including diabetes(McCune and Johns, 2002). Similar trend has been observed in the instruction of world health organization which favour traditional/alternate therapies for the effective management of diabetes, especially in those countries where fruitful results are coming from conventional therapies (Santha *et al*., 2006, Waheed *et al*.,

2006). The results of our study demonstrated profound attenuation of hyperglycemic effect in alloxan induced diabetic rabbits by the crude extract/fractions of *P. duthiei* after 24th day of treatment. The aqueous fraction was the most significant fraction among the extract/fractions tested followed by ethyl acetate fraction and thus revealing the polar nature of anti-hyperglycemic components.

The genus of this plant has already been showed anti-diabetic activity regulate glucose uptake in liver and muscles by way of PKB/Akt activation, restoring the intracellular energy balance (Lima *et al*., 2012)and strong possessed various phytochemical that could be responsible for the results of our study (Tsai *et al*., 1998, del Refugio Ramos *et al*., 2004). Nevertheless, *P. duthiei* has not been subjected to e isolation pure secondary metabolites. It, is therefore, suggested to explore this plant for the isolation of pure molecules to know the real chemical background of this study as well as to discovery new effective molecules of clinical uses.

In short, it is concluded that the extract/fractions of *P. duthiei* caused marked amelioration of hyperglycemic effect in alloxan induced and thus provided pharmacological rationale for the use of the plant as antidiabetic. However, further detail studies on the isolation of secondary metabolites are required to confirm the chemical nature of the constituents responsible for current show.

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