Effect of *Carthamus tinctorius* (Safflower) on fasting blood glucose and insulin levels in alloxan induced diabetic rabbits

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Abstract: Diabetes mellitus is a major threat to present and future generations. The role of herbal medication has emerged as a safe alternative to currently available medication due to its decreased potential to produce side effects, hence effect of *Carthamus tinctorius* was observed on fasting blood glucose and insulin levels in alloxan induced diabetic rabbits. Thirty five healthy male rabbits were divided into 5 groups with 7 rabbits in each (Normal control, diabetic control, diabetic treated with glibenclamide, diabetic treated with *Carthamus tinctorius* extract at doses of 200 and 300mg/kg of body weight). Drug and extract were given orally for 30 days and the values for blood glucose levels were observed after 15th and 30th day of treatment by using standard reagent kits provided by Human Germany. While insulin levels were checked at the end of the study by using Architect i1000 by Abbott Diagnostics USA. Animals were also observed for any gross toxicity during the study. Results revealed that *Carthamus tinctorius* has significant hypoglycemic effect at 200mg/kg and 300mg/kg doses as compared to diabetic control group. Insulin levels were significantly increased in Glibenclamide treated as well as *Carthamus tinctorius* treated groups as compared to diabetic control.

Keywords: *Carthamus tinctorius* (CT), Fasting blood glucose (FBS), Type 2 Diabetes Mellitus (Type 2 DM)

INTRODUCTION

Despite much advances in the treatment of type 2 diabetes mellitus, there is a great threat to the future generations since disease impart changes in life style and human behavior (Zimmet, 2001; Wild 2004). Once thought to be a disease of west, has now become a global health priority (Juliana, 2009). It is estimated by the international Diabetic federation that 6-4% (285 million people) were suffering from Diabetes in year 2010 and the figure will reach to 7.7%(439 million people)by the year 2030 (Shaw 2010) Pakistan stands at the seventh position in diabetes prevalence with 6.9 million being affected by the disease (Hayat and Shaikh, 2010).

Diabetes mellitus is a multifactorial chronic disease and has become a challenging job to treat with currently available drugs due to recurrent drawbacks leading to poor compliance (Earl, 2005). Most of the oral hypoglycemic agents are adaptogenic (Moller, 2001), helping to control hyperglycemia on one hand but tends to increase weight on the other hand hence worsening the condition (Kaleem *et al.,* 2008).

However increased research in herbal medicines has revealed many plants with hypoglycemic activities that have been reported to be safer alternatives in type 2 diabetes worldwide (Shukla *et al.,* 2000; Bhattaram *et al.,* 2002; Mahomed and Ojewole, 2003; Hou, Zhang 2005, Huang *et al.,* 2005, Kaur 2012).

Carthamus tinctorius, commonly known as safflower, false saffron or dyers saffron belongs to the family asteraceae and since centuries it was grown from China to Mediterranean regions (Weiss, 1971), but at presently is grown for commercial purposes in Pakistan, India, USA, Ethiopia, Mexico, Kazakhstan, Argentina, Australia, Spain, Turkey, Canada and Iran. Apart from yielding a yellow orange dye, high quality edible oil is also extracted from its florets rich in polyunsaturated fatty acids and is gaining popularity due to its medicinal value (Weiss, 1983; Parisa, Jinous and Mehrnaz, 2012).

MATERIALS AND METHODS

Plant material and extract preparation

The dried safflower (*Carthamus tinctorius*) flowers were obtained from a local herbal dealer in Peshawar and identified by the Professor Ghazala H Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. The dried safflower was soaked in ethanol for 8 weeks, then filtered and evaporated under reduced pressure in rotary evaporator at 40^{o}C, followed by freeze drying at -30^{o}C in HEJ research institute of

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chemistry. Approximately 10 gm of extract was yielded from 100 gm of the petals.

Animal selection and induction of diabetes

35 healthy male rabbits weighing between 1.4–1.8 kg were used in this study. The animals were housed in separate cages under 50 – 60% humidity and were provided with green leafy diet and water at labitum. All animals were divided in five groups with 7 animals in each group. One group served as a normal control, while diabetes was induced in animals of remaining groups after an overnight fast by intraperitoneal injection of freshly prepared aqueous solution of alloxan monohydrate at the dose of 150mg/kg (Gupta *et al.,* 2009).

Rabbits with fasting glucose levels more than 200mg/dl after 48 hours of alloxan administration were considered as diabetic and included in the study. The animals did not develop hyperglycemia i.e. fasting glucose > 200 mg/dl were replaced with new animals. All animals received saline, standard drug and extract according to following protocol

Group a: Normal control group received normal saline 10 ml/kg
Group b: Diabetic control received saline 10 ml/kg
Group c: Diabetic rabbits received 2.5mg/kg Glibenclamide for 30 days
Group d: Diabetic rabbits received 200mg/kg of CT extract for 30 days
Group e: Diabetic rabbits received 300mg/kg of CT extract for 30 days

Collection of samples

Five ml blood was collected in gel tubes through cardiac puncture technique on 15th and 30th day of dosing period. Samples were centrifuged at 3000rpm for 15 minutes in 14K Humax centrifuge machine. Blood glucose levels were analyzed on HumaLyser 3000 (semi-automatic chemistry analyzer by Human) using standard reagents supplied by Human Germany. Insulin levels were determined at the end of the study using Architect i1000 of Abbott Diagnostics, USA.

Data was analyzed by using SPSS v.16. All values were expressed as mean ± standard error to the mean and were compared with control values by one way ANOVA, P values <0.05 were considered statistically significant and <0.01 as highly significant.

RESULTS

Table reveals glucose lowering effect of CT extracts, as compared to diabetic control, normal control and glibenclamide treated animals. Fasting blood sugar (FBS) levels after day 15 were significantly decreased to 162.71 ± 3.72 mg/dl as compared to diabetic control at 200 mg/kg but was highly significantly reduced to 135.50 ± 9.02 mg/dl at 300mg/kg as compared to diabetic control group i.e. 250.50±25.05mg/dl, However after 30 days there was highly significant decrease in FBS to 118.28±9.34 and 104.50 ±4.63 mg/dl at both 200 mg/kg and 300mg/kg respectively as compared to diabetic control group i.e. 258.00±25.12 mg/dl. However FBS were reduced highly significantly to 132.85±8.61 mg/dl and to 106mg/dl both after 15 and 30 days in Glibenclamide treated rabbits as compared to the controls. Insulin levels were decreased in diabetic control rabbits but increased highly significantly to 99.85±1.06 µIU in Glibenclamide treated rabbits and significantly to 99.28 $\pm$ 1.11 µIU and 100.66 $\pm$ 3.61 at 200 and 300mg/kg dose of CT extracts as compared to control group i.e. 9.57 $\pm$ 0.97 µIU.

DISCUSSION

Sedentary life style and remarkable changes in dietary habits are major contributory factors in the development of type 2 DM, once developed exposes the patients to a situation which makes it a challengeable job for a clinician to treat due to micro and macro vascular complications as the disease advances (Derek 2001). Currently used oral hypoglycemic agents have some limitations regarding their safety and efficacy; hence there is a strong need to develop indigenous, inexpensive, crude or purified botanical alternative antidiabetic drugs (Vankatesh, 2003).

Nature has gifted us with a variety of herbs which can serve as better alternatives, thus the focus of research has been changed and now herbal medicine are widely investigated for their hypoglycemic and hypolipidemic actions due to low incidence of adverse effects and easy availability. In past few years literature reveals hundreds of studies in which herbal medicine have shown promising results, hence present study has been performed to investigate an agent which possesses all the characteristics of an ideal hypoglycemic agent and prevent the complications of type 2 DM which if left untreated may lead to damage and dysfunction of various organs (Lyra, 2006).

Carthamus tinctorius (CT) commonly named as Kusum in Pakistan and India, is also known as Safflower, false saffron or dyer's saffron. It belongs to the genus carthamus, specie tinctorius of the family asteraceae. Both petals and seeds are rich in many nutrients and are utilized for their medicinal properties in traditional medicine (Ahmed, 2010). More than four hundred plants with glucose-lowering effects have been explored and hundreds of polysaccharide have been reported (Ernst, 2000). Safflower has been used as Chinese herbal tea since centuries for its multiple health benefits. Nowadays CT is gaining popularity for its medicinal properties all

Table: Comparison of fasting blood glucose and insulin levels between control and treated groups

Groups	FBS (mg/dl) 15 days	FBS (mg/dl) 30 days	Insulin (μU/ml) Levels 30 days
Normal Control (n = 7)	91.28 ± 1.97	90.85 ± 3.28	9.57 ± 0.97
Diabetic Control (n = 4)	250.50 ± 25.05	258.00 ± 25.12	9.25 ± 1.7
G. Treated (n = 7)	132.85 ± 8.61**	106.57 ± 7.27**	99.85 ± 1.06**
CT 200mg/kg (n = 7)	162.71 ± 3.72*	118.28 ± 9.34**	99.28 ± 1.11**
CT 300 mg/kg (n = 6)	135.50 ± 9.02**	104.50 ± 4.63**	100.66 ± 3.61**

n=31, Values are expressed as Mean + SEM, *P ≤ 0.05 significant as compared to diabetic control.
**P ≤ 0.01 highly significant as compared to compared to diabetic control.

over the world, the extract of florets have been utilized for cardiovascular disease, swelling and pain associated with trauma and menstrual problems (Mcheward *et al.,* 2012).

In present study CT extract was evaluated for its blood glucose lowering effect and insulin levels in alloxan induced diabetic rabbits, results reveal to have blood sugar lowering effect, which were in accordance to Rahimi, 2009 and Mandade, 2012.

Insulin is essentially required for utilization of glucose hence deficiency of insulin cause hyperglycemia (Alam 2003 and Edwin, 2006). Present study reveals highly significant increase in insulin levels by CT at both doses after 30 days (table) as compared to diabetic control. Hence, it can be assumed that CT produce hypoglycemia possibly by increasing insulin secretion. The increase in insulin levels by CT might be due to anti-inflammatory effect of extract which ultimately resulted in decreasing the blood sugar levels (Wang, 2011).

CT possesses 20-30% w/w neocarthamin, cathamidin, carthanin, lignans and polysaccharides. It also contains carthamin which is known for its poly unsaturated fatty acids (linolenic acid 78%) flowers are also rich in vitamin A, iron, phosphorus and calcium (Nimbkar, 2002). Hence hypoglycemic effect of CT may also be due to high carthamin content in flowers of orange color used in present study.

Carthamin has highest antioxidant potential due to its polyunsaturated fatty acid (linolenic acid) which helps in free radical scavenging activity (Choi, 2012 and Han, 2010). CT suppresses the activation of free radicals and helps reducing the oxidative stress (Parisa *et al.,* 2012), which may ultimately be helpful in improving diabetes. Hence use of CT can safely be advocated both for the prevention and treatment of type 2 DM.

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