Hypoglycemic and hypolipidemic activities of crude seeds of *Centratherum anthelminticum* in healthy volunteers and type 2 diabetic patients

Hina Akram Mudassir[1]*, Shamim Akhter Qureshi[2], Muhammad Bilal Azmi[3], Muhammad Ahsan[3], Musab Kamran[1] and Sakina Jafar[1]

[1]Department of Biochemistry, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[2]Department of Biochemistry, University of Karachi, Karachi, Pakistan
[3]Department of Biochemistry, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan

Abstract: This study first time reports the hypoglycaemic activity of crude seeds powder (CSP) of *Centratherum anthelminticum* in healthy and type 2 diabetic volunteers. In addition, hypolipidemic effect of same CSP was also determined in healthy volunteers. Healthy individuals were divided into control and two test groups T_1 and T_2 treated with 200 & 400mg of CSP. Similarly, type 2 diabetic patients were also divided into positive control (PC) treated with metformin 600mg and two test groups DT_1 (CSP 400mg + metformin 600mg) & DT_2 (CSP 400mg). Each group has 6 individuals and each treatment was done orally. CSP 400mg was found more hypoglycaemic on all time intervals from 30 to 120min when oral glucose tolerance test was conducted in healthy volunteers. Both test quantities of CSP 200 & 400 mg were found successful in same healthy personsin decreasing the levels of triglycerides & total cholesterol (p<0.05), low & very low density lipoprotein cholesterols (p<0.01) and keeping the level of high density lipoprotein cholesterol as same as it was observed in control group. Similarly, CSP 400mg along with metformin and alone was also found helpful in lowering the fasting blood glucose levels in type 2 diabetic patients (DT_1 & DT_2) -24.99% and -20.62% respectively as compared to diabetic group only treated with metformin (PC), (p<0.01). Therefore, CSP of *C. antheminticum* proves effective hypoglycaemic and hypolipidemic agent by possibly inducing glucose tolerance in healthy individuals and type 2 diabetic patients.

Keywords: CSP, type 2 diabetes, hypoglycaemia, *C. anthelminticum.*

INTRODUCTION

Hyperglycaemiaoccurs due to insulin resistance or lack of insulin. It is the most prevailing form of metabolic disorder in both developed and developing countries because of less active daily routine (Halban *et al*., 2014, Kahn *et al*., 2014). Type 2 diabetes encompassed85% whereas type 1 and gestational diabetes attributed to 15% (Guariguata *et al*., 2014). Treatment of diabetes mostly done by medicines that either reduce insulin resistance (like metformin, thiazolidinedione) orenhance its secretion (like sulfonylurea, incretin) but long term usage of these medicines bring critical side-effects, so encouragement towards herbal remediesis needed that can also lower postprandial hyperglycaemic response for managing diabetes (Kahn *et al*., 2014).

Centratherum anthelminticum Kuntze (kali zeeri) is a member of *Asteraceae* and its seeds are rich in many phytochemicals especially polyphenols, flavonoids, alkaloids, etc (Shamim *et al*., 2016; Ngeh and Rob, 2013; Amir and Koay, 2011). Its prominent flavonoids include tetrahydroxy chalcone, tetrahydroxy flavone and butin (Guilian *et al*., 2004). Whereas quercetin glycoside, naringenin-7-O-glucoside and kaempferol are reported with antidiabetic, antioxidant, and anti-inflammatory effectiveness (Paydar *et al*., 2013). The seeds also showed antimicrobial activities (Lei *et al*., 2012). Customarily these seeds are popular as astringent, anthelmintic, diuretic, tonic, etc to treat fever, skin diseases, kidney stones, hiccough, intestinal gripes, and eye infections (Lei *et al*., 2012; Singh *et al.*, 2012; Galani and Panchal, 2014).

Ethanol seeds extract of *C. anthelminticum* was reported to improve insulin secretion and sensitivity in fructose induced type 2 hyperglycaemic rabbits (Mudassir and Qureshi, 2015). Therefore, the purpose of this study was to investigate the potential of crude seeds powder of *C. anthelminticum* against glycaemic response in human volunteers and type 2 diabetic patients plus its effect on lipid profile in healthy volunteers.

MATERIALS AND METHODS

Crude seeds powder of C. anthelminticum (CSP)

The seeds of *C. anthelminticum* were bought from local market and kept as (KU/BCH/SAQ/02) after identification. The seeds were cleaned from dirt and grounded into powder with a help of blender before use.

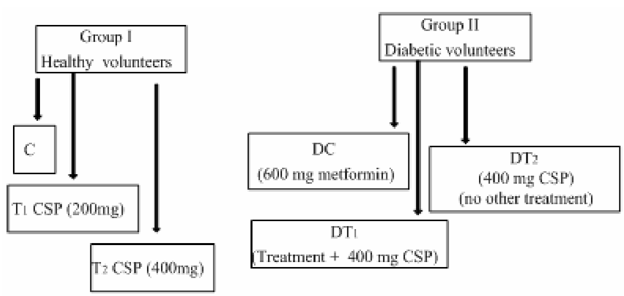
**Corresponding author:* e-mail: hina28577@yahoo.com

Type 2 diabeticand healthy volunteers

Eighteen (18) type 2 diabetic patients (male and female 30-45 years) were randomly selected in this study, having no history of other complications except hyperglycaemia. Of which, twelve of them were on antidiabetic medicine whereas other six were regulated their hyperglycaemia through diet. Similarly,18 healthy volunteers were selected for performing oral glucose tolerance test (OGTT).All healthy and diabetic volunteers were asked for fasting of about 10-12 hours before starting the experimental procedure. The study was done with the approval of Institutional Review Board (IRB) of FUUAST (Certificate Reference No: 2002) along with a written informed consent from all the participants.

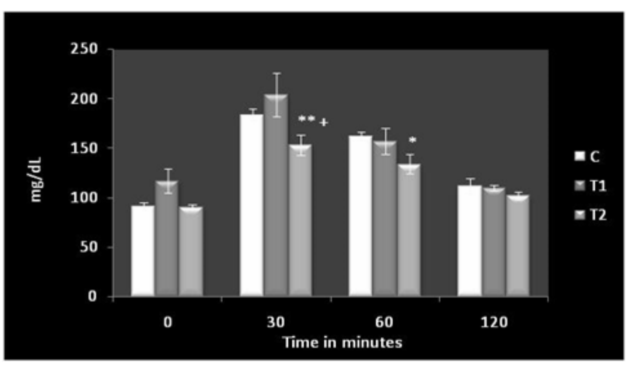
Determination of hypoglycemic activity and serum lipid profile

Overnight fasted healthy volunteers and diabetic type 2 patients (group II) were divided into two groups including healthy normal group I and diabetic group II (6 subjects/group) according to the fig. 1.



[C: control, PC: positive control, D: diabetic, T: test, CSP: crude seeds powder]

Fig. 1: Grouping of healthy & diabetic volunteers.



Values are mean ±SD n=6 +p <0.01 when compared between test groups, *p<0.05 when compared to control group

Fig. 2: OGTT of CSP of *C. anthelminticum* in healthy volunteers.

Group I(C, T_1& T_2) was assigned for OGTT. After the fasting of 10-12 hours each individual in these groups allowed to take 73gm glucose in approximately 200 ml of water then volunteers other than control were advised to take their allocated treatment immediately after glucose. After the induction of hyperglycemia plus treatment, blood glucose levels were monitored at 0, 30, 60 and 120 minutes with the help of glucometer by pricking the finger tips of participants. After 3 hours blood samples of the participants belonged to C, T_1& T_2groups were drawn and sera were separated for the biochemical assessment of lipid profile including triglycerides (TG), total cholesterol (TC), high, low and very low density lipoprotein cholesterols (HDL-c, LDL-c & VLDL-c).

Likewise with a little difference fasting blood glucose level of Group II (PC, DT_1& DT_2)was monitored prior (0 hr) to their allocated treatments then after the oral intake of their treatments, blood glucose level was monitored on same time intervals in same manner.

Physical & biochemical parameters

The percent glycemic change between control and treated groups was calculated by using the following formula (Azmi and Qureshi, 2012).

Percent glucose reduction = $[(G_x - G_o) / G_o] \times 100$

Where G_o = mean blood glucose level of normal (C) group in healthy volunteers and positive control (PC) group in diabetic patients at decided time intervals

G_x= mean blood glucose levels of test groups of healthy volunteers and diabetic patients at same time intervals respective to their control.

Parameters like serum TG, TC & HDL-c were done by kit methods (Randox, United Kingdom) while LDL-c, and VLDL-c were calculated by the given formulae (Friedewald *et al*., 1972).

LDL-c (mg/dl) = TC – (TG /5) – HDL-c

VLDL-c = TG (mg/dl) /5

STATISTICAL ANALYSIS

Results are recorded as mean ±SD. Data evaluation was done by one-way analysis of variance (ANOVA) using SPSS 17. Differences between control and test groups were considered significant at p<0.05 and p<0.01.

RESULTS

Effect of CSP of C. anthelminticumon glucose tolerance and its effect on lipid profile in healthy volunteers

CSP 400 mg in OGTT decreased significant blood glucose level (p<0.05) in test group T_2 (Group I) as compared to control after 30 & 60 min whereas the same group T_2 also showed significant decrease in same parameter at 30 min (p<0.01) as compared to test group T_1 treated with CSP 200mg (fig. 2). The percent glycemic reduction in T_1 was maximum after 60 min as -3.08% whereas in T_2 it was observed from -8.92 to -17.28% from 30-120 min (fig. 3).

Similarly, CSP 200 & 400 mg in healthy volunteers in T_1 & T_2 (Group I) showed a significant decrease (p<0.01) in the values of serum LDL-c and VLDL-c by keeping the level of HDL-c almost same in their respective groups when matched with control group whereas serum TG and TC in (T_1 & T_2) showed a significant decrease (p<0.05) and (p<0.01) respectively (fig. 4).

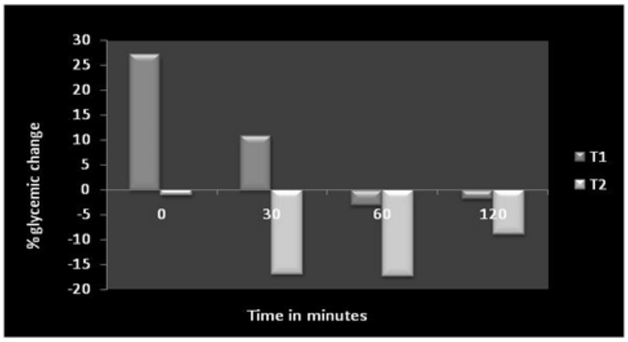
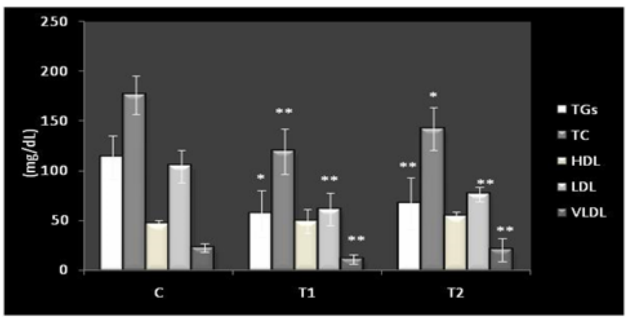


Fig. 3: Effect of CSP of *C. anthelminticum* on percent glycemic change healthy volunteers



values are mean ±SD n=6**p <0.01, *p<0.05 when compared to control group

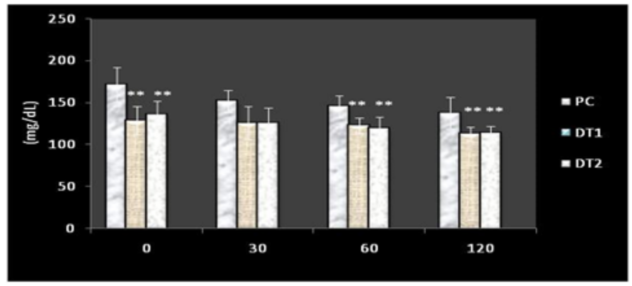
Fig. 4: Effect of CSP of *C. anthelminticum* on Lipid profile in healthy volunteers

Effect of CSP of C. anthelminticumon glucose tolerance in type 2 diabetic patients

The CSP 400 mg produced significant hypoglycaemic effect (p<0.01) after 0, 60 and 120 min in DT_2as same as CSF 400mg along with metformin 600mg in DT_1 (group II)when compared both these groups with PC (fig. 5) and showed percent glycaemic reduction from -24.99 to -18.11% and -20.62 to -17.57% respectively in DT_1 and DT_2 from 0 to 120 min (fig. 6).

DISCUSSION

The chief characteristics of type 2 diabetes is either relative insulin shortage or insulin resistance that can be explained as a decreased insulin mediated glucose uptake by peripheral tissues and excessive hepatic glucose production, results in impaired or persistent hyperglycaemia. Individual's ability to tolerate glucose can be predicted by assessing postprandial blood glucose level either by blood sugar tolerance test in normal and after meal intake in diabetic patients where healthy individuals show normal blood glucose level within 120 min because of the presence of insulin which converts excess glucose into glycogen in liver whereas the blood glucose level remains high in diabetic patients even after 120 min.



Values are mean ±SD n=6 **p<0.01 when compared to positive control group

Fig. 5: Effect of CSP of *C. anthelminticum* on Fasting blood glucose in type 2 diabetic patients

Fig. 6: Effect of CSP of *C. anthelminticum* on Percent glycemic reduction in type 2 diabetic patients

*C. anthelminticum*is well-famous for its number of medicinal properties in Asian countries like Pakistan and its neighbours, many of which have been investigated in animal models so far like its antidiabetic activity infructose-induced type 2 rabbit model has been published (Mudassir and Qureshi, 2015, Fatima *et al.*, 2010). The present study first time reports the hypoglycaemic activity of seeds powder of *C. anthelminticum* in healthy individuals and type 2 diabetic patients. The seeds powder in a dose of 400mg/kg was found hypoglycaemic on all time intervals from 0to 120 min in OGTT by decreasing the blood glucose level from -8 to -17% in its respective test group and proved to induce glucose tolerance in healthy volunteers. In addition, both test doses (200 & 400mg/kg) of seeds powder of *C. anthelminticum* also showed significant hypolipidemic activity in same healthy individuals by decreasing the levels of bad lipids (TG, TC, LDL-c & VLDL-c) and keeping the good lipid (HDL-c) assame as it was found in control group.

Induction of glucose tolerance by *C. anthelminticum* seeds powder was also supported by observing its antidiabetic activity in type 2 diabetic patients. Where same seeds powder in a dose of 400mg/kg was evident in reducing the fasting blood glucose level in both diabetic test groups treated with seeds powder alone (DT_2) and along with metformin (DT_1) by showing prominent percent reduction in same parameter from 0 to 120 minin comparison to type 2 diabetic (positive control) group treated with only metformin 600mg.

The antidiabetic action of *C. anthelminticum* may be due to either of the following possible mechanisms, like by decreasing glucose absorption in gastrointestinal tract *via* inhibiting the activity of carbohydrate hydrolyzing enzymes including α-amylase and α-glucosidase. An *in-vitro* study was already reported the inhibition of these two enzymes by *C. anthelminticum* (Ani and Naidu, 2008; Amir and Chin, 2011). Inhibition of α-amylase and α-glucosidaseis one of the critical therapeutic strategies used to control/manage type 2 diabetes.

by increasing insulin secretion from functional beta cells of pancreas. An *in-vitro* study reported the action of chloroform fraction of methanolic seeds extract of *C. anthelminticum* in accelerating insulin release from beta-TC6 cell line (Arya *et al*., 2012).

By inhibiting the hydrolysis of TGs, redistributing the fatty acids in adipose tissues and removing them from circulation thereby decreasing the insulin resistance and making the receptors available for insulin binding or action. Ethanolic seeds extract of *C. anthelminticum* was reported to decrease insulin resistance in fructose-induced type 2 diabetic rabbit model (Mudassir and Qureshi, 2015).

byinhibiting the process of hepatic gluconeogenesis by obstructing the action of two rate- regulatory enzymes (glucose 6-phosphate and phosphoenol pyruvate carboxykinase) of the same pathway in liver. Metformin, the known antidiabetic medicine, is also adopting the same mechanism of action in decreasing blood glucose level in type 2 diabetic patients (Arya *et al.*, 2012). Similarly, few medicinal plants like *Zingiber officinale* are reported to inhibit the process of hyperglycemia (Daily *et al*., 2015).

In the end, the hypoglycaemic activity of seeds powder of *C. anthelminticum* may be resides in its phytochemicals like polyphenols, flavonoids, alkaloids which are already reported in these seeds extract.

CONCLUSION

The present study provides evidence that *C. anthelminticum* seeds powder promotes glucose metabolism and produces antihyperglycemic effect in healthy and type 2 diabetic patients. However, to understand its exact mechanism of action, long term investigations are required in type 2 diabetic patients.

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