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Centratherum anthelminticum seeds administration improved risk factor markers in metformin treated type 2 diabetic patients

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Abstract: This study was designed to assess the effect of *Centratherum anthelminticum* seeds administration on blood and serum risk factor markers in type 2 diabetic (T2D) patients using metformin. It was a randomized non-blinded, controlled, 3month trial. T2D patients were registered from Sindh Government Hospital New Karachi, Karachi. There were 3 groups, DT (Diabetic Test, n=20), DC (Diabetic Control, n=20) HC (Healthy Control n=20, healthy individuals of similar age were selected). DC (metformin 500mg), DT (capsule of *C. anthelminticum* seeds powder CAPca + metformin 500mg each) and HC (CAPca 500mg) were given daily. Fasting blood glucose, body weights were monitored weekly while blood samples were collected on first and final day of the trial for biochemistry. Administration of CAPca showed significant decrease in % glycaemia ($p<0.0001$) and % Hb glycosylation ($p<0.05$) values. Serum risk factor markers including direct bilirubin & uric acid ($p<0.01$), creatine kinase & alanine aminotransferase ($p<0.0001$) and creatinine ($p<0.05$) were reduced significantly. CAPca administration improved blood and serum markers in healthy volunteers too. These improved blood & serum markers in T2D patients indorsed synergistic effect of CAPca with metformin to manage secondary challenges of T2D.

Keywords: *Centratherum anthelminticum* seeds powder, type 2 diabetes (T2D), fasting blood glucose, HbA1c, inflammatory markers.

INTRODUCTION

High blood glucose and mitochondrial superoxide assist each other in type 2 diabetes, this partnership is manifested in non- enzymatic condensation of hemoglobin that produce oxidative stress and other secondary complications (Asmat *et al*., 2016). The frequency of insulin resistance type 2 diabetes in south Asian countries is getting high and Pakistan had 0.033 billion people with T2D in the year 2022 (Azeem *et al*., 2022), still increasing. Oxidative stress and CVDs are the head and tail of T2D originated from metabolic stress in the form of free radicals via high NADPH-oxidase activity linked to high levels of protein kinases. The major insulin resistant cellular dysfunction occurs in hepatocytes, adipocytes and myocytes where hindered performance of nitric oxide synthase trigger oxidation lead to secondary challenges (Yaribeygi *et al*., 2019), increased 50% death rate (Rehman and Akash, 2017).

Healthy diet routine and body weight are the important adjustments in T2D along with anti-diabetic drug like metformin but its long term usage brings biochemical alterations in T2D patients (Baker *et al*., 2021). Herbs with anti-diabetic phytochemicals is a new strategy to cure secondary challenges and about 73% diabetic individuals use anti-diabetic herbs in addition (Li and Weng *al*., 2017). Phenols and flavonoids containing phyto-medicine are ROS scavengers, compete with glucose transporters and digestive enzymes hence used in treating T2D and due to low cost and lesser side effects plant based medicines would become a safer choice (Alam *et al*., 2022).

The seeds of the plant in *Asteraceae* family called *Centratherum anthelminticum*, named as kali zeeri has registered as a significant sugar lowering agent along with persistence anti-inflammatory activities due to the presence of phenolic acids, steroids, fatty acids and terpenes (Dogra *et al*., 2020). Its polyphenolic content like quercetin glycoside, kaempferol, caffeic and decanoic acid etc. promoted sugar utilization and insulin secretion (Shoaib *et al*., 2023; Arya *et al*., 2012). These seed in different solvents extract also reported to increase glucose influx and its energy metabolism by sensitizing GLUT-2 and GLUT-4 transporters in diabetic animal models (Arya *et al*., 2012). Previously ethanol extract of these seeds (at different dosages) also reported to inverse insulin resistance, improve hyperlipidemia, HbA1c glycation and antioxidant enzymes levels in insulin resistant animal model (Lateef and Qureshi, 2013; Mudassir *et al*., 2018a; Mudassir *et al*., 2018b). Hexane oil fraction of these seeds

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recently reported to improved nephron function in renal model of diabetic animal (Baig *et al*., 2022).

The main purpose of this work was to explore the effect of *C. anthelminticum* seeds (capsules) administration in T2D patients in combination with metformin in terms of risk factor markers investigation in blood and serum and to evaluate its role in the management of secondary challenges.

MATERIALS AND METHODS

Centratherum anthelminticum seeds (Herbarium No. KU/BCH/SAQ/02) were bought from local market and cleansed then grinded into fine powder for capsulation process (mixing up with magnesium stearate as lubricant 250mg/capsule) done from Mektum Homoeo Pharma and termed as CAPca in this work. Research Ethics Committee (FUUAST) in collaboration with Sindh Government Hospital New Karachi (Hospital Review Board) approved this work (Reference # 2032 & 560). This study was a non-blinded randomized controlled trial. Forty T2D volunteers (age between 40-60 years) from Sindh Government Hospital New Karachi (Karachi) were enrolled to this study with their written consents (3 months, October - December, 2019) under the observation of diabetes experts. The inclusion criteria for T2D participants was diabetic type 2 history of 5 years and HbA1c value less than 9%, with metformin treatment. The exclusion criteria were with no other disorders like type 1 diabetes, hypertension, mental illness, liver diseases and Tuberculosis. Twenty healthy individuals were inducted of same age group. The selected volunteers were distributed into three groups (20 participants/gp) i.e.

Diabetic Test (DT, metformin and CAPca 500mg each)
Diabetic Control (DC, metformin 500mg)
Healthy Control (HC, CAPca 500mg)
All groups were given their dosages two times daily. Volunteers visited to General physicians (diabetes care unit, New Karachi Hospital) on weekly basis where their body weight (bw) and fasting blood glucose (fbg, using glucometer) were monitored. The biochemical investigations were done on the first and last day of trial i.e. blood samples were drawn two times for laboratory procedures.

Percent change in body weight (%BW), HbA1c glycosylation (% HbA1c) and percent fasting glycaemia (%FG) of all groups were evaluated by using the given formula respectively
% value = (Final day record - Initial day record) / (Initial day record) x 100

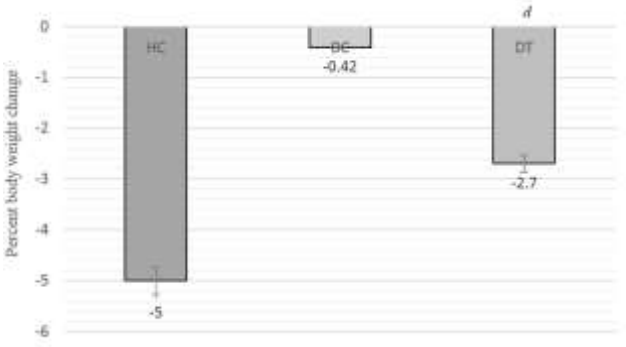
Blood (HbA1c) and serum risk factor markers including alanine aminotransferase, creatinine, uric acid, total bilirubin and direct bilirubin (ALT, CK, TB, Cr, UA) were determined by HPLC method (Bergmann and Sypniewska, 2016) and using Randox kits (UK). Indirect Bilirubin (IB) was estimated through the formula given as IB= TB - DB

STATISTICAL ANALYSIS

Results are expressed as mean ±SD (standard deviation) and considered as significant at $p<0.05$, $p<0.01$, $p<0.0001$ when analyzed by one-way ANOVA (SPSS version 18).

RESULTS

The DT showed a significant reduction in body weight ($p<0.05$), fasting blood glucose and HbA1c ($p<0.0001$) values as compared to DC (table 1). In individual groups significant improvement was observed in HC & DT in body weight ($p<0.05$), fasting blood glucose and HbA1c ($p<0.0001$) values, reduced significantly than their first records (table 1). CAPca functional response was also displayed in the significant improvement of percent body weight and percent glycemic changes in DT, both showed prominent reduction ($p<0.0001$) (-2.7 body weight change & -42% glycemic change) after 3 months as compared to DC (fig. 1 & 2). Likewise, the percent HbA1c glycosylation value was also significantly reduced ($p<0.05$) (-0.2%) in DT than DC (fig. 2). Interestingly HC also showed improvements in all these percentage changes values confirming CAPca activity (fig. 3).

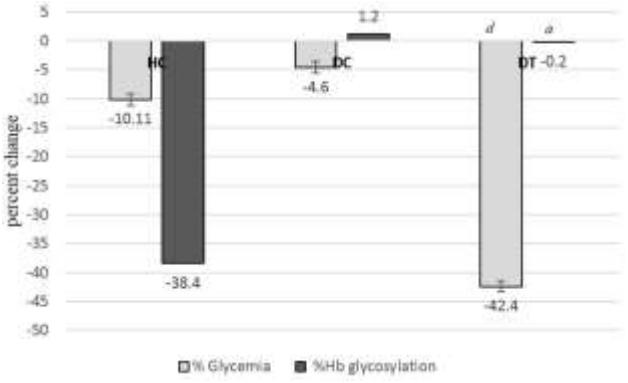


Superscript ^{d} shows statistical significance (ANOVA) when compared with DC Abbreviations: HC healthy control, DC diabetic control, DT diabetic test.

Fig. 1: Effect of CAPca administration on percent body weight change (%BW) in diabetic and healthy volunteers.

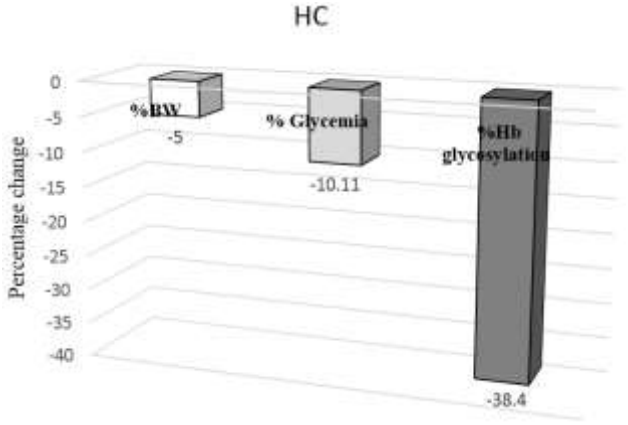
When compared with DC, CAPca usage in DT showed improvements almost in all serum risk factor markers i.e. serum direct bilirubin (DB), uric acid (Ua) values were significantly ($p<0.01$) reduced than DC (table 2). Similarly, serum creatine kinase (CK), alanine aminotransferase (ALT) levels ($p<0.0001$) and serum creatinine (Cr) level ($p<0.05$) were also reduced significantly than DC. (table 2). In individual groups, DT showed significant ($p<0.0001$) reduction in serum levels of TB, DB CK Ua ($p<0.0001$) and serum levels of ALT

and Cr (p<0.01) than its first records. Similarly, HC showed significant (p<0.0001) reduction in serum bilirubin (TB, DB, IB) and creatine kinase (CK) levels than its 1st records whereas serum uric acid (Ua) value was reduced (p<0.01) significantly. (table 2). The metformin using group DC showed significant reduction only in serum direct bilirubin (DB) and creatine kinase (CK) levels (p<0.01 & p<0.0001) than its 1st records (table 2).



Each bar represented as mean ± SD, n= 20, ^{a}p<0.05, ^{b}p<0.01, and ^{d}p<0.0001, when compared with DC Superscripts^{a,b,d} show statistical (ANOVA) significance Abbreviations: HC healthy control, DC diabetic control, DT diabetic test

Fig. 2: Effect of CAPca administration on percent change in blood risk factor markers in diabetic and healthy volunteers.



Each bar represented percentage change as mean, n= 20, Abbreviation: HC healthy control, BW body weight, Hb hemoglobin

Fig. 3: Effect of CAPca administration on percentage changes in body weight & blood risk factor markers in healthy volunteers.

DISCUSSION

Metformin is classified as synthetic antidiabetic drug formerly extracted from plant source (*Galega officinalis*) (Usai *et al*., 2022) though its prolong use is convoyed with metabolic stresses specially on heart, kidney and liver function but recently its efficiency has reported to be complimented with herbal medicine in lowering sugar in T2D patients (Prasopthum *et al*., 2022; Valdes *et al*., 2023). Local herbs are nature´s gift, mostly possess distinct bioactive compounds (alkaloids, terpenes, flavonoids etc.) (Srivastava *et al*., 2014) that play the key role in metabolism like act as carbohydrates enzymes inhibitors (glucosidase, amylase) as well as stimulate pancreatic insulin synthesis with muscular & adipocyte glucose utilization (Mudassir *et al*., 2018b; Laila *et al*., 2022).

There is number of mechanisms of antidiabetic action of *C. anthelminticum* seeds reported earlier like by inhibiting sugar hydrolyzing enzymes α-amylase and α-glucosidase (due to polyphenols, flavonoids, alkaloids presence), enhancing insulin release from pancreatic beta cells (Ani and Naidu, 2008; Amir and Chin, 2011) likewise chloroform fraction of seeds (*in-vitro*) improved insulin release from beta TC6 cell line (Arya *et al*., 2012). Another study reported that its ethanol extract improved insulin resistance in fructose-induced type 2 diabetic rabbit model (Mudassir and Qureshi, 2015) via hepatic gluconeogenesis inhibition and glucose 6-phosphate and phosphoenol pyruvate carboxykinase (rate- regulatory enzymes) block, interestingly metformin also exhibited the similar mechanisms of action in the management of blood glucose level in T2D patients (Arya *et al*., 2012).

This work showed CAPca in combination with metformin synergistically fit in normalizing almost all selected blood and serum markers in T2D patients. As these seeds reported to be a potent glycolytic agent (rich source of flavonoids that exhibited antiglycation activity closer to rutin) in diabetic models (Mudassir *et al*., 2018a; Shoaib *et al*., 2023), it can be predicted that this synthetic-herbal drug combination complimenting each other in the form of α-glucosidase inhibition to reduced hepatic glucose uptake where metformin presence already suppressed gluconeogenesis and this way reverting hyperglycemia (i.e. ↑glucose utilization) that also stable HbA1c values and reduced free radical production in these patients (fig. 2). In this study CAPca also showed significant contribution in normalizing physical fitness (in terms of body weight) as well as blood glycaemia and HbA1c glycosylation (table 1, fig. 2) than only metformin usage, validating its restorative quality (activate GLUT-4 transporter in gut, liver and muscles) (Arya *et al*., 2012; Mudassir *et al*., 2018a). The HbA1c glycation and body weight resistance features of CAPca was also previously described in terms of anti-hyperlipidemic property where these seeds enhanced liver lipases linked lipid metabolism (Mudassir and Qureshi, 2015), that helped to unmask insulin receptors and improved percentage values of fasting glycaemia and glycosylation (Qureshi *et al*., 2016; Mudassir *et al*., 2018b) so as observed in this study (fig. 2). The healthy heart retrieving ability of CAPca was seen in improved serum CK level (CVD markers of diabetes) in DT group (table 2) even the other groups (DC & HC).

Table 1: Effect of CAPca administration on body weight and blood risk factor markers in diabetic and healthy volunteers

Groups	Body weight (kg)		Fasting blood glucose(mg/dl)		HbA1c (mg%)	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
HC	55 ±4.0	52.25 ±2.9$^{\#}$	89 ±9.1	80 ±10.13^{+}	5.2 ±0.47	5 ±0.33^{+}
DC	79 ±12.24	79.33 ±12	195 ±30.89	186 ±36.1	8.3 ±1.18	8.4 ±1.5
DT	75 ±12.07	73 ±10$^{a\#}$	198 ±57.87	114 ±28.81^{+d}	8.35 ±1.43	6.8 ±0.67^{+d}

All values are mentioned as mean ± SD, $^{a}p<0.05$, $^{b}p<0.01$ and $^{d}p<0.0001$, when compared with DC, $^{\#}p<0.05$ & $^{+}p<0.0001$ when individual group compared with its initial (1st day) record, Superscript$^{a,b,d,\#,+}$shows statistical significance Abbreviation: HC healthy control, DC diabetic control, DT diabetic test, Hb hemoglobin

Table 2: Effect of CAPca administration on serum risk factor markers in diabetic and healthy volunteers

Serum markers	HC		DC		DT	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
Total bilirubin TB (mg/dl)	0.96±0.18	0.86±0.17^{+}	0.47±0.06	0.5±0.52	0.56±0.11	0.54±0.07^{+}
Direct bilirubin DB (mg/dl)	0.23±0.05	0.20±0.04^{+}	0.17±0.16	0.16±0.01*	0.19±0.23	0.15±0.008^{+e}
Indirect bilirubin IB (mg/dl)	0.72±0.18	0.66±0.17^{+}	0.30±0.06	0.35±0.07	0.35±0.17	0.39±0.07
Cretinine Cr (mg/dl)	0.70±0.20	0.76±0.18	1.05±0.24	1.05±0.15	0.97±0.14	0.92 ±0.10^{*d}
Uric acid Ua (mg/dl)	4.45±1.16	3.76 ±0.73*	4.81±0.84	5.36±0.53	5.37±1.30	3.82 ±0.80^{+e}
Creatine kinase CK (U/L)	27.33±13.9	15.45±5.33^{+}	66.16±11.37	55±13.09^{+}	71.85±9.04	44.40±7.81^{+f}
Alanine aminotransferase ALT (U/L)	16.0±3.52	21 ±4.81	38.00±8.31	38.16±5.11	41.40±9.54	29.95±6.39^{*f}

All values mentioned as Mean ±SD, $^{d}p<0.05$, $^{e}p<0.01$ and $^{f}p<0.0001$, when compared with DC, $^{\#}p<0.05$, $^{*}p<0.01$ & $^{+}p<0.0001$ when individual group compared with its initial (1st day) record, Superscripts$^{d,e,f,\#,*,+}$ show statistical significance, Abbreviations: HC healthy control, DC diabetic control, DT diabetic test

Showed this marker within reference range (Mudassir *et al*., 2018a) (table 2). Hepatocyte membrane instability is secondary to constant hyperglycemia that can be read in terms of serum ALT, bilirubin (total, conjugated/ unconjugated) levels (Ahamed *et al*., 2021) (table 2). Hence CAPca showed free radical fighter, in combination with metformin it brought back the healthy endothelial function by improving risk factor markers (serum ALT, uric acid, creatinine and bilirubin) (Shoaib *et al*., 2023) in DT than single metformin cure (table 2).

Therefore, these findings provided us to design a novel herbal treatment using *C. anthelminticum* seeds that will help to address second-rate challenges of insulin resistance diabetes and will provide a good insulin stimulation (with no side effects) than single metformin cure.

CONCLUSIONS

C. anthelminticum seeds can be a potential future antidiabetic medicine because in many previous animal and clinical models due to their potent antioxidant phyto-chemistry in metabolic disorders specially diabetes. These herbal seeds normalize high blood glucose and its related parameters in T2D by almost same way as metformin does but for finding the exact mechanism our next scheme of work will to analyze how the natural elicitors of these seeds compliment the synthetic drug chemistry (as metformin does, seen in current work) and to find out if any adverse effects will be seen or not, in a well-designed clinical trial with safety measures.

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