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Original Article

Hypoglycemic Effect of Dichloromethane Extract of *Caeselpinia bonducella* leaves in Rats with High Fructose Diet Induced Diabetes

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Abstract

Insulinresistancehasbeen considered as the most important component of type 2 diabetes mellitus (DM2). Plants used infolkmedicine to treat diabetes mellitusre present aviable alternative forthe control ofthis disease. This study was aimed to examine the antidiabetic effects of dichlorome thane extract of *Caesalpinia bonducella* in an animal model of DM2. Diabetes was induced in male Wistarrats (6-8 week sold) by feeding 21% fructose in drinking water for 8 weeks. They were treated with dichlorome thane extract of leaves of *Caesalpinia bonducella* (DCM 200, DCM 400) for 2 weeks. After diabetes induction and the last day of the experiment, body weight, fasting blood glucose, plasmainsulin, urinevolume and urine glucose were assayed. Blood glucose, plasmainsulin, urine gluco sean durin evolume were in creased significantly after 8 weeks of high fructose feeding (P<0.005); DCM 200 reduced body weight, plasma insulin andurineglucose (p<0.05)and no significant difference in blood glucose and urine volue. While DCM 400 significantly decreasedblood glucose (p<0.05), urineglucose (p<0.01) and plasma insulin (p<0.01), body weight (p<0.05)andurine volume (p<0.05) when compared with that of 8th week values. The study showed hypoglycemia effects and improvement of insulin resistance of dichloromethane extract of leaves of *Caeslpinia bonducella*. These result scan bee xtra polated to human sand these extracts might be useful in the treatment of insulin resistance.

Keywords: Hypoglycemia; Insulin Resistance; Fructose; *Caesalpenia bonducella.*

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Introduction

The current trend suggests that there is a substantial consumption of fructose per capita as a sweetener in the food industry, primarily in the form of sucrose (a disaccharide consisting of 50% fructose) and high-fructose corn syrup (HFCS; 55–90% fructose content) [1].

It is widely accepted that excessive intake of fructose can promote metabolic changes such as hyperlipidemia, hyperinsulinemia, insulin resistance, hyperuricemia, hypertension, glucose intolerance and non-enzymatic fructosylation of proteins resulting in advanced glycated end products and associated complications [2,3]. In addition, excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus, non-alcoholic fatty liver disease and cardiovascular diseases [4].

Studies have reported that rats fed with a high-fructose diet form a model of diet-induced insulin resistance, associated with hyperinsulinemia, hypertriglyceridemia and glucose intolerance [5]. Feeding of a high fructose diet to normal Wistar rats provides a dietary model of type 2 diabetes associated

with insulin resistance, hyperinsulinemia, hypertriglyceridemia [1] andhypertension [6].The precisemolecular mechanisms that high fructose diet induces the abnormalities in liver carbohydrate metabolism are not fully understood. Thus, fructose has been implicated as the useful tool to induce insulin resistance in animals [7]. Recently, antioxidants are found to be effective in preventing metabolic disturbances and resultant diseases induced by high-fructose diet [8,9].

Caesalpiniabonducella(L.) Fleming (Syn. *Caesalpinia bonduc* (L.)Roxb, Syn. *Caesalpinia crista*Linn.), belonging to the family Fabaceae /caesalpiniaceae, is a prickly shrub widely distributed all over the world specially, in India, Sri Lanka and Andaman and Nicobar Islands, in India specially found in tropical regions [10,11]. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in traditional system of medicine [12]. The plant has been reported to possess anxiolytic, antinociceptive, antidiarrhoeal, antioxidant, hypoglycemic, antidiabetic, antimicrobial, antiproliferative, antiestrogenic, antimalarial, antitumor, antipsoriatic, larvcidal and antifilarial activities [13]. Phytochemical analysis of seeds of *Caesalpiniabonducella* has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids [14,15].

Materialsand Methods

Plan Collection and Processing

Leaves of *C.bonducella*were collected from Rajkot (Gujarat). The plant was identified by comparing it morphologically and microscopically with description given in different standard texts and floras. The plant was identified and authenticated by prof. VISHAL MULIYA, Christ College, Rajkot and a voucher specimen was deposited. The leaves material was cleaned and dried in shade, powdered and stored in air tight container at room temperature.

Plantmaterial& Preparationof Extracts

The powdered leaves wereextracted using dichloromethane by Soxhlet extractionat controlled temperature (40 °C) for 72 hours. Resulting solutions were filtered through Whatman filter paper (No.42). The filtrates so obtained were concentrated in a water bath at low temperature (40 °C). Later, dichlorome thane was evaporated off and dry the residue in vacuum oven to remove solvent completely from extract. The extracts so prepared were subjected for further studies.

Preliminary Phytochemical Investigation

All four crude extracts were subjected to qualitative phytochemical screening for the presence of various secondary metabolites [16].

Animals

Male Wistar rats(6-8 weeks old weighing approximately 170–200g) were housed in stainless steelcages (three animals per cage). The animals were kept 1 week prior to the experiment for acclimatization in an air-conditioned animal room (22±2^{0}C) under a 12 hlight/darkcycle with free access to standard pellet diet and water. The study was approved by the ethics committee, and the rats were maintained in accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Induction of Hyperglycemia

21% Fructose solution was used as inducing agent for induction of hyperglycemia and insulin resistance [17].

Experimentaldesign

The animals were randomly divided into four groups containing six rats in each group as given below: Group I – Normal Control (NC) Received 0.5% CMC, po, and normal drinking water, group II – Diseases Control (DC) received 0.5% CMC, po, and 21%fructose solution as drinking water, group III – (DCM – 200) Received 200 mg /kg body weight dichloromethane extract of *Caesalpinia bonducella* leaves, po, and 21% fructose solution as drinking water, group IV – (DCM – 400) received 400 mg /kg body weight dichloromethane extract of *Caesalpiniabonducella* leaves, po, and 21% fructose solution as drinking water.

All animals group were given 21% fructose solution as drinking water for 8 weeks. After 8 weeks of fructose feeding, animals with fasting serum glucose greater than 135 mg/dl were considered as diabetic and received investigational drug treatment. From the 9th week, drug interventions were initiated for next 2 weeks. Body weight of animals was measured at weekly intervals. Extracts were suspended in 0.5% CMC and administered by gastric gavage tube once a day for 2 weeks and rats were fed standard pellet diet throughout the study.

Sample Collection

From overnight fasted animals, blood samples

were collected from retro-orbital plexuses on 0, 56th (8th week), 70thday(10th week) under the influence of light ether anesthesia and subjected to centrifugation at 6000 rpm for 15 min to obtain serum.

Biochemical Measurements

Fasting glucose and insulin levels were measured in serum using span diagnostics kit, Surat, Gujarat, India and Insulin ELISA kit, Kamiya Biomedical Company, USA respectively. Similarly urine samples were collected and urine volume was measured at 6 hr intervals on 0, 56th and 70th day and urine glucose levels were measured using span diagnostic kit, Surat, Gujarat, India.

Statistical Analysis

Values are given as mean ± standard error of mean (SEM). The statistical analysis of biochemical parameters were conducted using prism 5.03. Within-group comparisons were performed by paired test. In all analyses, *P*<0.05 was considered significant.

Result

Preliminary Phytochemical Investigation

Qualitative phytochemical screening of different crude extracts of *Caesalpiniabonducella* (Table 1) showed the presence of secondary metabolites such as alkaloids, glycosides, saponins, flavonoids, triterpenoids, phytosterols, phenolic compounds/ tannins, in variable proportions.

Effects of *C.Bonducella* on Body Weight, Blood & Urine Glucose, Serum Insulin and Urine Volume

Values for blood glucose, urine glucose, plasma insulin and urine volume increased significantly ininduced diabetes groups (DC, DCM-200, DCM-400) after 8 weeks of high fructose feeding (P<0.05), while no significant difference was observed in these parameters in NC group during this period of time (Table 2 & 3). In the NC group in the 10th week no significant differences was observed in the studied parameters compared to those of 8th week, however in DC group the changes were no table. The mean difference of body weight of rats in DC group was 126.36g (p<0.001) and 158.94g (p<0.001) after 8 and 10 weeks of fructose feeding, respectively when compare with 0 day values and the difference was significant.

At the end of 10th week (Table 2 & 3), no significant difference was detected inblood glucose and urine volume in the DCM 200 group,while body weight(p<0.05), urine glucose(p<0.01) and plasma insulin(p<0.05)showed significant decline when compared with that of 8th week values. Whereas, DCM 400 significantly decreased blood glucose (p<0.05), urine glucose(p<0.01) and plasma insulin (p<0.01), body weight(p<0.05)and urine volume (p<0.05) when compared with that of 8th week values.

Table 1: Extract yield* of *C. Bonducella* leaves in dichloromethane

Class	
% *Yield	7.5
Alkaloid	+
Carbohydrates	-
Phytosterols	+
Tannins	+
Proteins & Amino Acids	-
Flavanoids	+
Glycosides	+
Saponins	+
Gum/Mucilage	-

*Yield (%) is a percentage of the weight of the extract in relation to the weight of the raw material. +: Present, -: Absent

Table 2: Effects of *C.Bonducella* on body weight, blood & urine glucose, serum insulin and urine volume

GROUP (n=6)	Body Weight (g)			Serum Insulin (ng/ml)			Urine volume (ml)		
	0	8	10	0	8	10	0	8	10
NC	178.68±5.39	235.13±6.6≠	254.46±6.83ns	1.07±0.06	1.62±0.09≠	1.61±0.07ns	3.57±0.37	2.73±0.09*	3.07±0.21ns
DC	181.56±3.87	307.92±7.69Φ	340.50±7.72*	1.15±0.05	2.75±0.06Φ	2.92±0.14ns	2.83±0.16	7.38±0.34Φ	7.27±0.32ns
DCM 200	175.89±4.84	312.06±7.96Φ	286.38±6.22*	1.19±0.05	2.70±0.07Φ	2.50±0.07*	3.12±0.19	7.05±0.35Φ	6.12±0.33ns
DCM 400	175.49±4.22	315.57±10.24Φ	268.54±10.17*	1.13±0.10	2.79±0.06Φ	2.17±0.05≠	3.28±0.22	6.63±0.38Φ	5.22±0.43*

Values are expressed as Mean±SEM and 8th week values were compared with 0 day values and 10th week values were compared with 8th week value using paired t test.ns= not significant,*= P<0.01,≠= P<0.05,Φ= P<0.001NC: Normal control, DC: Diease control, DCM 200 & 400: Dichloromethane extract of C.bonducella 200 & 400 mg/kg body weight

Table 3: Effects of *C.Bonducella* on body weight, blood & urine glucose, serum insulin and urine volume

GROUP (n=6)	Urine Glucose (mg/dl)			Blood Glucose (mg/dl)		
	0	8	10	0	8	10
NC	NA	NA	NA	96.98±3.70	88.20±4.04^{ns}	93.55±4.47^{ns}
DC	NA	53.62±5.78	48.16±6.39	98.48±2.31	138.78±3.45$^{\Phi}$	147.34±6.71$^{\Phi}$
DCB 200	NA	55.21±6.45	21.35±3.29$^{\neq}$	97.05±3.31	133.48±4.76$^{\neq}$	122.47±6.91$^{\Phi}$
DCB 400	NA	54.19±7.90	8.27±1.89$^{\neq}$	95.79±3.48	140.98±4.20$^{\neq}$	107.37±8.43*

Values are expressed as Mean±SEM and 8th week values were compared with 0 day values and 10th week values were compared with 8th week value using paired t test. NA= Not Applicable, ns= not significant, * = P<0.01, ≠ = P<0.05, Φ = P<0.001

Discussion

Primary treatment goals in diabetes include restoration and maintenance of normogly caemia, avoidance of diabetic complications and prevention of cardiovascular events.Inadditiontoglycemic control, management of hyper insulinemia is also essential for limiting the complications of type 2 diabetes mellitus(Type 2 DM) [18].

Fructose is a potent reducing sugar that promotes the formation of toxic advanced glycation end-products. Excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus, and non-alcoholic fatty liver disease characterized by an impaired glucose tolerance test. In the present study clearly shows, high fructose feeding resulted in significant increase in the body weight, fasting hyperglycemia, hyperinsulinemia and urine glucose leading to the development of insulin resistance.

Chronic fructose feeding in experimental animals is reported to produce glucose tolerance and increase in body weight associated with hyperinsulineamia and loss of normal *in vivo* sensitivity to insulin [19,20]. Our results are consistent with previous studies which found that consumption of high-fructose diets markedly induces an increase in body weight, glycemia associated with hyperinsulinemia.

Exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis with the accumulation of triglycerides (TGs), which contributes, in turn, to reduced insulin sensitivity and hepatic insulin resistance / glucose intolerance [21]. Animal studies have shown that high fructose diet-fed rats display hepatic insulin resistance and altered lipid metabolism due to hepatic lipid accumulation as a result of the burden of fructose metabolism [22]. This cluster of disorders is similar to those observed in human multimetabolic syndrome or syndrome X or insulin resistance syndrome, which is observed in prediabetic patients that progresses to type 2 diabetes mellitus and cardiovascular diseases [23].

In the present study *C.bonducella*treatment significantly reduced the body weight, plasma glucose, urine glucose, urine volume and insulin level. This therapeutic effect could be attributed to the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids[24,25]. Parameshwar *et al* and Mandal *et al* have already reported the antioxidant and reactive oxygen species scavenging activity of Methanolic extract of*Caesalpiniabonducella* leaf. The antioxidantswith ROS scavenging ability may have great relevance in the prevention of diabetes linked oxidative stress [26,27]. The antihyperglycemic action of the extracts may be due to blocking of glucose absorption [28]. The aqueous ethanolic seed extract of *C.bonducella* has shown hypoglycemic activity in streptozotocin induced diabetic rats and produced increase in secretion of insulin. Moreover, the phytochemical of *C.bonducella*- Bargenin, caesalpinine A, α and β amyrinlupeol have demonstrated increase in insulin release from pancreatic cells [29]. Liu IM *et al* have reported that *Abelmoschusmoschatus*stimulates insulin-signalling cascades and improves insulin-signalling transduction by modification of Ser/Thr phosphorylation of IRS-1 [30]. In addition, *Brassica juncea,* Brassicaceae and *Calotropisgigantean,* Apocynaceae restored the pre-diabetic state of insulin resistance [31,32] in rats fed fructose-enriched diet. Besides, *Tinosporacordifolia* Menispermaceae has been found to activate various hepatic enzymes such as hexokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6bisphosphatase, and glucose-6-phosphate dehydrogenase [33,34] and significantly ameliorated diabetic state in high-fructose diet rats. Previously published reports also suggest that bonducin (a Homoisoflavone) [35] has a potential to improve insulin resistance. The plant under investigation is also reported to have bonducin as one of the phytochemicals. Therefore the observed insulin sensitivity improving effect of *C. bonducella* in present study could be due to bonducin.

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