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Phytochemical Investigation And Anti-Diabetic Activity Study Of The Plant Of Cleome Rutidosperma

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Abstract

The hypoglycaemic and antihyperglycaemic effects of metha-nol extract of leaves of Cleome rutidosperma (Cr) DC (Family: Capparidaceae) was investigated in Wistar rats. Fifty normo-glycaemic male rats (120 g–200 g) were divided into groups A (hypoglycaemic study; n = 20) and B (antihyperglycaemic study; n = 30). Each experiment had one control group and three groups administered with Cr (100, 200 or 400 mg/kg) respectively. Group B had two additional groups of diabetic-untreated rats and glibenclamide-treated diabetic rats. Diabe-tes was induced in Group B rats (except control) fasted over-night for 12 h by intraperitoneal injection of Alloxan (100 mg/kg). Fasting blood glucose levels (FBGL) were determined and alloxan-treated rats with BGL > 200 mg/dl 48 h post-induction were considered diabetic. Data obtained were analyzed using One-way ANOVA and Duncan Multiple Range Test ($p < 0.05$). Cr-treated rats showed significant decline in BGL with notewor-thy decline by day 3 post-treatment at the dose of 200 mg/kg (236.40 ± 14.72 mg/dl) from 336.40 ± 21.06 mg/dl. Cr at the dose of 200 mg/kg (72.20 ± 6.18 mg/dl, 69.20 ± 7.81 mg/dl, 137.80 ± 7.15 mg/dl and 70.60 ± 10.66 mg/dl) showed better gl ycemic control compared to glibenclamide (194.50 ± 7.75 mg/dl, 253.75 ± 7.20 mg/dl, 284.25 ± 10.56 mg/dl and 156.00 ± 10.80 mg/dl). Cr-treated rats also showed pro-gressive weight gain through the course of the study. This study demonstrated Cr has antihyperglycemic effect with more rapid onset of action and better glycemic control compared to glibenclamide.

Keywords: Cleome Rutidosperma , Alloxan, Diabetes, hyperglycemia..

Introduction

Over the years, awareness of the efficacy of herbal remedies for treatment of various disease conditions has increased. Diabetes mel-litus (DM), one of such conditions, characterized by metabolic de-rangement of glucose metabolism leading to chronic hypergylcae-mia has been traditionally managed with medicinal plant prepara-tions [1]. Diabetes has been classified into two broad types viz; Type 1 diabetes (insulin-dependent diabetes mellitus, IDDM or juvenile diabetes) which is due to an autoimmune destruction of the insulin producing pancreatic beta cells [2] and Type 2 diabetes (non insu-lin-dependent diabetes mellitus, NIDDM or adult-onset) caused by increased insulin

resistance in cells [3]. A third transient type is rec-ognized as gestational diabetes which occurs during pregnancy [4].

The principal hormone regulating the uptake and metabolism of glucose from the blood into most cells of the body, particularly liver, muscle, and adipose tissue is insulin. Central to development of DM is insulin deficiency or insensitivity of peripheral insulin re-ceptors [5, 6]. Glucose is made available to the body from food ab-sorbed in the gastrointestinal tract by glycogenolysis or gluconeo-genesis. Insulin inhibits these biochemical processes or stimulates the transport of glucose into fat and muscle cells, and the storage of glucose in the form of glycogen [3]. Beta cells (Î²-cells) in the is-lets of Langerhans of the pancreas secrete and release insulin into the blood in response to rising levels of blood glucose as seen in post-prandial hyperglycaemia. The hormone glucagon, acts in the opposite fashion to insulin [7].

Persistent hyperglycaemia leads to complications in other organs such as the kidneys and the eyes. The kidneys reach a threshold of glucose reabsorption, thus glycosuria ensues [8]. The osmotic pressure of urine is increased, inhibiting further reabsorp-tion of water by the kidneys leading to more urine production (polyuria) and increased fluid loss. The lost blood volume is replaced osmotically from body water and other body compart-ments, causing dehydration and polydipsia [3]. Well managed blood glucose level has been shown to reduce organ/system effects, complications and their severities in diabetic patients. The major aim of DM therapy is targeted at maintenance of blood glucose as close to normal without causing hypoglycaemia, usu-ally by healthy diet, exercise, weight loss, and use of appropriate medications. Insulin is required in the case of Type 1 DM, Type 2 DM is managed with oral medications possibly in conjunction with insulin therapy [9].

Numerous medicinal plants are also employed for management of DM in West Africa, as in other regions of the world. One of such medicinal plants is Cleome rutidosperma (Family: Capparidaceae), commonly known as fringed spider flower or purple Cleome, a na-tive of Tropical Africa and found as garden weed in Asia and Aus-tralia. The leaves are prepared as hot water infusion or chewed whole for management of DM. The anti-inflammatory, analgesic, antipyretic, antioxidant and free radical scavenging effects of C. rutidosperma were reported by Bose et al. [10, 11]. The antiplas-modial, anthelminthic, wound healing and hypoglycaemic effects of the roots of C. rutidosperma [12–14], and the aerial parts were reported to possess antioxidant, diuretic and laxative effects [14– 16]. A dearth of information exists on the hypoglycaemic and antihyperglycaemic effects of the leaves which are used for man-agement of diabetes. This study was therefore designed to inves-tigate these effects of in normoglycaemic and alloxan-induced hy-perglycaemic Wistar rats at dose of 100, 200 and 400 mg/kg, p.o. respectively.

Materials and Methods

Plant preparation

Fresh leaves of Cleome rutidosperma (Cr) were harvested from local area . It was identified at the Department of Botany, University of odisha and a voucher specimen was collected (Voucher Number UIH-22548). The leaves were picked off the stems, air dried and pulverized with a mortar and pestle. The grounded leaves were macerated in meth-anol (96 %) for 72 h and afterward decanted. The filtrate was con-centrated using a rotary evaporator and tharea of e extract obtained was stored at 4 °C. Fresh extract was reconstituted daily according to the doses required.

Experimental animal

Male Wistar rats were obtained from and housed at the Animal House, Department of Veterinary Physiology, Biochemistry and Pharmacology, odisha. The rats were fed with commercially available pelletized rat ration and allowed access to feed and clean water ad libitum. Only normoglycaemic rats were included in the study from the start point. Fifty normo-glycaemic male Wistar strain albino rats (120 g–200 g) were divid-ed into two groups of twenty for hypoglycaemic and thirty for hyperglycaemic study.

Hypoglycaemia study

Twenty normoglycaemic rats (125–150 g) were randomly and equally divided into four groups of five rats each. Rats in group 1 served as the control for this experiment. Cr extract was adminis-tered to rats in groups 2, 3 and 4 at doses of 100, 200 or 400 mg/kg respectively. The rats were fasted overnight (12 h) before the commencement of the hypoglycaemic study and the fasting blood glucose levels were determined. The extract was administered, rats were fed and blood glucose levels (BGL) were monitored using a glucometer (AccuChek active®) at 1, 2, 3, 6, 12 and 24 h post-feed-ing with commercially available rat pellets to determine the hypo-glycaemic effect of Cr.

Hyperglycaemia study

Thirty normoglycaemic rats were randomly distributed into six sub-groups (Groups 1, 2, 3, 4, 5 and 6). Group 1 served as control nor-moglycaemic rats. Diabetes mellitus was induced in Groups 2-6 rats by a single intraperitoneal injection of alloxan monohydrate (100 mg/kg). Group 2 was administered with Glibenclamide (0.07 mg/kg), Groups 3, 4 and 5 were administered orally with Cr at doses of 100 mg/kg, 200 mg/kg or 400 mg/kg, while Group 6 rats were hyperglycaemic but untreated throughout the study. Marked increase in blood glucose levels post-administration of alloxan were indicative of induction of T2DM (17). Rats with BGL ≥ 200 mg/dl were considered diabetic 48 h post-administration of alloxan. The extract or glibenclamide commenced and the post-prandial BGL monitored at 1, 2, 3, 6, 12 and 24 h post- administration of Cr and glibenclamide and also on days 1, 2, 3, 5, 7, 10 and 14 of the study.

Statistical analysis

Data was presented a mean ± Standard Error of Mean (SEM), ana-lyzed using One-way analysis of variance (ANOVA) and statistical significance was determined using Duncan Multiple Range at $p < 0.05$.

Results

Normoglycaemia study

The methanol leaf extract of Cleome rutidosperma (Cr) caused no reduction in the blood glucose level (BGL) in normoglycaemic rats. The BGL of the three groups were comparable to those of rats in the control group (▶Table 1).

Table 1 Blood glucose levels of normoglycaemic rats administered with methanol extract of Cleome rutidosperma leaf observed within 24 h post- administration.

Group	FBG	1Hr	2Hr	3Hr	6Hr	12Hr	24Hr
Ctrl	100.0 ± 3.79	115.80 ± 1.26	115.19 ± 1.26	114.30 ± 0.56	106.12 ± 1.90	100.10 ± 0.63	76.5 ± 6.01
100	87.67 ±	93.67 ± 9.76	105.33 ±	126.20 ±	118.67 ±	92.67 ± 6.70	86.67 ± 1.44

mg/kg	4.30		8.26	3.58	3.36		
200 mg/kg	75.00 ± 2.68	94.67 ± 0.93	95.67 ± 1.37	103.60 ± 0.45	109.00 ± 9.18	85.33 ± 2.73	70.67 ± 2.98
400 mg/kg	83.33 ± 1.37	96.67 ± 1.69	113.00 ± 2.79	105.67 ± 0.68	114.00 ± 6.20	102.67 ± 1.13	77.67 ± 2.46
Diab- Diabetic rats; Gliben – Glibenclamide							

Hyperglycaemic study

BGL of diabetic rats administered with all doses of Cr (100 mg/kg, 200 mg/kg or 400 mg/kg) fluctuated through the course of the in-itial 24 h post-administration of Cr (▶Table 2). Significant decline in the BGL were observed for rats administered with extracts of Cr from day 3 post-administration. Rats administered with Cr at dose of 100 mg/kg and 200 mg/kg exhibited further decline in their BGL from 281.00 ± 9.90 mg/dl and 236.40 ± 14.72 mg/dl to 192.67 ± 14.73 mg/dl and 72.20 ± 6.18 mg/dl respectively for days 3 and 5. Concurrently on days 3 and 5, further fluctuations were observed in rats administered with Cr extract at the dose of 400 mg/kg and glibenclamide, the standard antidiabetic agent ad-ministered.

In the overall, BGL of rats administered with Cr extract exhibit-ed significant decline compared to the diabetic but untreated rats through the course of treatment on days 3, 5, 7, 10 and 14. By day 5, BGL of rats administered with Cr at doses of 200 mg/kg (72.20 ± 6.18 mg/dl) was comparable to that of control normogly-cemic rats (97.75 ± 4.66 mg/dl), although a slight rise in BGL was observed on day 10 (137.80 ± 7.15 mg/dl), but this resolved by day 14 with BGL of 70.60 ± 10.66 mg/dl (▶Table 3).

Diabetic rats administered with glibenclamide showed gradual decline in BGL from 1 hour post-treatment levels of 348.75 ± 9.86 mg/dl to 252.25 ± 14.56 mg/dl (24 h), 194.50 ± 7.75 mg/dl (day 5) and 156.00 ± 10.80 (day 14). The ex-tract of Cr at doses of 200 mg/kg showed a more rapid and signifi-cant decline in BGL from pre-treatment (336.40 ± 11.06 mg/dl) to 1 hour post-treatment (282.20 ± 8.65 mg/dl), 24 post-treatment (271.60 ± 11.02 mg/dl), day 5 (72.20 ± 6.18 mg/dl) and day 14 (70.60 ± 10.66 mg/dl) (▶Table 2 and 3). Changes in the weight of the rats showed that the diabetic untreated rats significantly lost weight through the course of the study, from 167.6 ± 5.4 g (day 1) and 154.6 ± 4.2 g (day 14). However, the diabetic rats treated with the extract of Cleome rutidosperma at doses of 200 mg/kg and 400 mg/kg showed significant weight gain by day 14 (172.8 ± 9.7 g and 170.8 ± 3.5 g) (▶Table 4).

Discussion

In this study, results obtained showed that methanol leaf extract of Cleome rutidosperma (Cr) did not have hypoglycemic effect. Blood glucose levels (BGL) of the normoglycemic rats administered with the methanol extract of Cr were within range of normal val-ues in the 24 h post-prandial observation period. However, Cr caused significant ($p \leq 0.05$) reduction in the BGL of alloxan-in-duced diabetic Wistar rats, notably from day 3 of treatment. Fol-lowing the destruction of the β-cells of the Islet of Langerhans which secrete insulin by a single intraperitoneal administration of alloxan monohydrate (100 mg/kg), BGL of rats included in the an-tihyperglycemic study was elevated to > 200 mg/dl [17–19].

Table 2. Blood glucose levels of alloxan-induced diabetic rats administered with methanol extract of Cleome rutidosperma leaf observed with 24 hr post administration

Group	FBG	48HR	1HR	2HR	3HR	6HR	12HR	24HR
Control	69.00±10.41	79.50±0.65	87.25±4.19	88.50±9.17	86.25±7.06	76.25±1.89	71.75±2.66	67.25±5.36
Diab Ctr	74.33±5.90	267.67±11.82	398.33±8.28	432.00±7.05	438.67±66.06	450.33±14.69	548.33±15.32	426.00±10.96
Diab+100mg/kg	82.00±10.26	394.00±9.55	435.00±9.60	373.67±8.29	402.67±8.24	339.00±9.62	453.00±7.27	466.33±18.70
Diab+200mg/kg	57.60±1.36	336.40±11.06	282.20±8.65	267.20±18.71	271.20±7.58	278.20±8.30	258.20±5.05	271.60±7.02
Diab+400mg/kg	55.20±7.29	288.80±12.08	355.80±10.42	538.20±11.08	553.40±24.45	495.80±2.69	498.10±2.80	480.00±21.02
Diab+Gliben	78.75±2.14	295.25±16.80	348.75±9.86	339.00±12.45	344.50±9.28	374.25±9.86	507.50±6.81	252.25±14.56

Diab- Diabetic rats; Gliben – Glibenclamide

Diabetes mellitus (DM) is a metabolic disorder characterized by persistent derangement in glucose metabolism and glycemic con-trol is the hallmark of management of DM due to the devastating sequel of the disease [20]. In the event of persistently high BGL, the kidneys reach a threshold of reabsorption and glucosuria occurs. Urine osmotic pressure increases and inhibits reabsorption of water by the kidneys, leading to polyuria and increased fluid loss [8]. De-hydration and polydipsia result from the compensatory replace-ment of lost fluid with water held in body cells and other compart-ments [3]. Polyuria, polydipsia and weight loss are some clinical signs that accompany this metabolic disease [21]. A major contri-bution is made by hyperglycemia to the development and progres-sion of micro- and macrovascular complications, which include cer-ebrovascular, retinopathy, neuropathy, nephropathy and cardio-vascular diseases [22, 23].

Glucose is obtained mainly from intestinal absorption of food, the breakdown of stored glycogen from the liver and gluconeogen-esis, the generation of glucose from non-carbohydrate substrates [28]. Insulin functions to maintain glucose levels in the body, which may involve inhibition of the breakdown of glycogen or the process of gluconeogenesis and or facilitation of glucose transport into fat and muscle cells and its conversion to glycogen [6].

In response to increasing BGLs such as observed post-prandial, insulin is synthesized and released into the blood by beta cells (β-cells) of islets of Langerhans in the pancreas. In the event of hy-poglycemia, glucagon is released to mobilize glucose from its stor-age sites and stimulate gluconeogenesis [7]. Insulin insensitivity or resistance develops when insufficient insulin is released or the insulin itself is defective with poor cellular absorption of glucose by cell for energy production or incorporation into storage cells for later use. The overall effect is hyperglycemia, poor protein synthe-sis, and other metabolic derangements such as acidosis [3].

Diabetic but untreated rats remained hyperglycemic through the course of the study, while the BGL of extract treated rats pro-gressively declined to normal BGL. Varied levels of BGL were ob-served within the initial 24 h post-treatment of the diabetic rats with the methanol extract of Cr, with significant ($p < 0.05$) decline by day 3 post-treatment up to day 14. The antihyperglycemic ef-fect was more profound than that observed for rats administered with glibenclamide and Cr had a more rapid onset of action, espe-cially at the dose of 200 mg/kg. Rats treated with the dose of 200 mg/kg showed the best glycemic control in this study.

Table 3 Blood glucose levels of alloxan-induced diabetic rats administered with methanol extract of Cleome rutidosperma leaf observed within 14days of administration.

Group	DAY 1	DAY 3	DAY 5	DAY 7	DAY 10	DAY 14

Control	67.25 ± 5.36	84.25 ± 5.66	97.75 ± 4.66	85.00 ± 5.72	94.25 ± 3.75	99.50 ± 6.70
Diab Control	426.00 ± 9.96	235.00 ± 13.30	320.33 ± 10.51	422.33 ± 10.91	397.33 ± 8.97	326.67 ± 9.30
Diab+100 mg/kg	466.33 ± 8.70	281.00 ± 9.90	192.67 ± 14.73	191.67 ± 8.60	195.33 ± 15.60	179.67 ± 10.73
Diab+200 mg/kg	271.60 ± 11.02	236.40 ± 14.72	72.20 ± 6.18 *	69.20 ± 7.81 *	137.80 ± 7.15	70.60 ± 10.66 *
Diab+400 mg/kg	480.00 ± 10.02	258.40 ± 8.51	270.80 ± 19.32	373.00 ± 8.90	143.00 ± 12.07	189.80 ± 11.86
Diab + Gliben	252.25 ± 14.56	234.50 ± 13.97	194.50 ± 7.75	253.75 ± 7.20	284.25 ± 10.56	156.00 ± 10.80

* Indicates significantly ($p<0.05$) lower blood glucose level compared to control on the same column; Diab- Diabetic rats; Gliben – Glibenclamide

Table 4 Changes in weight of diabetic rats (n = 5) administered with methanol extract of Cleome rutidosperma in the course of a 14-day study.

Group	DAY 1	DAY 4	DAY 8	DAY 10	DAY 12	DAY 14
Control	171.0 ± 8.2 *	174.7 ± 8.5	178.0 ± 9.1	179.3 ± 8.4	181.0 ± 8.8	182.33 ± 8.3 *
Diab Control	167.6 ± 5.4^	163.5 ± 4.3	160.2 ± 3.2	156.2 ± 2.3	153.8 ± 2.9	154.6 ± 4.2^
Diab + 100 mg/kg	207.5 ± 5.9	209.8 ± 3.9	211.5 ± 4.6	208.4 ± 3.3	205.2 ± 4.6	203.9 ± 2.9
Diab + 200 mg/kg	164.0 ± 9.3 *	160.8 ± 9.7	162.2 ± 9.6	164.4 ± 9.4	165.2 ± 8.9	172.8 ± 9.7 *
Diab + 400 mg/kg	147.0 ± 3.1 *	150.4 ± 3.1	151.2 ± 2.4	152.0 ± 2.7	164.2 ± 2.4	170.8 ± 3.5 *
Diab + Gliben	188.3 ± 2.3	183.3 ± 3.8	186.7 ± 4.7	190.0 ± 3.2	189.0 ± 3.9	183.3 ± 5.9

* Indicates significant weight gain compared to day 14 on same column; ^Indicates significant weight loss compared to day 14 on same column; Diab- Diabetic rats; Gliben – Glibenclamide

Typically, DM is associated with chronic weight loss [29], which was also exhibited in this study. However, rats administered with the leaf extract of Cr showed progress increase in weight gain comparable to that observed for control rats. This weight loss has been associated with structural protein degradation and muscle wasting brought about by the process of gluconeogenesis from non-glu-cose stores [30].

Some medicinal plants are reported to exert hypoglycemic ac-tion by potentiation of insulin action, either via stimulation of insu-lin secretion from the pancreas or its release from bound insulin [31]. Some others act via extra pancreatic mechanisms by inhibi-tion of hepatic glucose production or

corrections of insulin resist-ance [32]. Although the probable mechanism of antihyperglycemic action of Cr leaf is unknown, it may be elucidated with further study. This study confirms that the traditional use of Cleome rutidosper-ma for management of diabetes mellitus is justified. Further study is warranted to determine its toxicology profile and isolate the bio-active principle responsible for the antihyperglycemic effects.

References

[1] Sharma K, Pareek A, Chauhan EK. Evaluation of hyperglycemic and hyperlipidemic mitigating impact of hibiscus Rosa sinensis (gudhal) flower in Type II diabetes mellitus subjects. Int J Appl Biol Pharmaceut Technol 2016; 7: 223–228

[2] American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2009; 32: (Suppl 1): S62–S67

doi:10.2337/dc09-S062

[3] Masharani U, German MS. Greenspan's basic & clinical endocrinology. 9th ed. Gardner DG, Shoback D. (eds) 2011 Chapter 17: New York: McGraw-Hill Medical; ISBN 0-07-162243-8

[4] Vambergue A, Fajardy I. Consequences of gestational and pregesta-tional diabetes on placental function and birth weight. World J Diabetes 2011; 2: 196–203

[5] Porte D, Baskin DG, Schwartz MW. A Critical Role in Metabolic Homeostasis and disease from C. elegans to humans. Diabetes 2005; 54: 1264–1276 doi: 10.2337/diabetes.54.5.1264

[6] Tuomi T, Santoro N, Caprio S et al. The many faces of diabetes: A disease with increasing heterogeneity. Lancet 2014; 383: 1084–1094

[7] Zhu X, Wallman J. Opposite effects of glucagon and insulin on compensation for spectacle lenses in chicks. Invest Ophthalmol Vis Sci. 2008; 50: 24–36 doi:10.1167/iovs.08-1708

[8] Hahr AJ, Molitch ME. Management of diabetes mellitus in patients with chronic kidney disease. Clinical Diabetes and Endocrinology 2015; 1: 2 doi:10.1186/s40842-015-0001-9

[9] Inzucchi SE, Bergenstal RM, Buse JB et al. Management of hyperglyce-mia in type 2 diabetes: A patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). 2012;

[10] Bose A, Mondal S, Gupta JK et al. Analgesic, anti-inflammatory and antipyretic activities of the ethanolic extract and its fractions of Cleome rutidosperma. Fitoterapia 2007; 78: 515–520

[11] Bose A, Mondal S, Gupta JK et al. Antioxidant and free radical scavenging activities of Cleome rutidosperma. Oriental Pharmacy and Experimental Medicine 2008; 8: 135–145 doi:10.3742/

OPEM.2008.8.2.135

[12] Bidla G, Titanji VPK, Joko B et al. Antiplasmodial activity of seven plants used in African folk medicine. Indian J. Pharmacol. 2004; 36: 245–246

[13] Mondal S, Dash GK, Acharyya S et al. Hypoglycaemic activity from the roots of Cleome rutidosperma DC. Biomed 2009; 4: 64–69

[14] Mondal S, Dash GK, Bal SK. Anthelmintic activity of Cleome ruti-dosperma DC. roots. Indian Drugs 2009; 46: 47–49

[15] Bose A, Mondal S, Gupta JK et al. Studies on diuretic and laxative activity of ethanolic extract and its fractions of Cleome rutidosperma aerial parts. Pharmacognosy Mag 2006; 2: 27–31

[16] Chakraborty AK, Charde MS, Roy H et al. Comparative study of antioxidant activity between ethanolic and aqueous extract of Cleome rutidosperma. Int J Pharmaceut Sci Res 2010; 1: 112doi: 10.13040/IJPSR.0975-8232.1(11).112-16

[17] Adeyi AO, Idowu BA, Mafiana CF et al. Rat model of food-induced non-obese-type 2 diabetes mellitus: comparative pathophysiology and histopathology. Int J Physiol Pathophysiol Pharmacol. 2012; 4: 51–58

[18] Kikumoto Y, Sugiyama H, Inoue T et al. Sensitization to alloxan-induced diabetes and pancreatic cell apoptosis in acatalasemic mice. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 2010; 1802: 240–246

[19] Oyedemi SO, Adewusi EA, Aiyegoro OA et al. Antidiabetic and haematological effect of aqueous extract of stem bark of Afzelia africana (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pac J Trop Biomed. 2011; 1: 353–358

[20] Bloomgarden ZT. Type 2 Diabetes in the Young: The evolving epidemic. Diabetes Care 2004; 27: 998–1010

[21] Cooke DW, Plotnick L. Type 1 diabetes mellitus in pediatrics. Pediatr Rev. 2008; 29: 374–384 quiz 385 doi:10.1542/pir.29-11-374 PMID 18977856

[22] Shim U, Lee H, Oh JY et al. Sleep Disorder and Cardiovascular Risk Factors among Patients with Type 2 Diabetes Mellitus. Korean J. Intern. Med. 2011; 26: 277–284

[23] Hamdy SM. Effect of Morus alba Linn extract on enzymatic activities in diabetic rats. Journal of Applied Sciences Research 2012; 8: 10–16

[24] Palanduz S, Ademoglu E, Gokkusu C. Plasma antioxidants and type 2 diabetes mellitus. Pharmacology 2001; 109: 309–318

[25] Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003; 17: 24–38

[26] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216–226

[27] Lodovicia M, Giovannellia L, Pitozzia V et al. Oxidative DNA damage and plasma antioxidant capacity in type 2 diabetic patients with good and poor glycaemic control. Mutation Research 2008; 638: 98–102

[28] Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 2005; 65: 385–411 doi:10.2165/00003495-200565030-00005 PMID 15669880

[29] Espeland M, Bahnson JL, Wagenknecht L et al. Reduction in Weight and Cardiovascular Disease Risk Factors in Individuals With Type 2 Diabetes: One-Year Results of the Look AHEAD Trial. Diabetes Care 2007; 30: 1374–1383 doi: 10.2337/dc07-0048

[30] Okoro IO, Umar IA, Atawodi SE et al. Antidiabetic effect of Cleome rutidosperma Dc and Senecio biafrae (Oliv. & Hiern) extracts in streptozotocin-induced diabetic rats. Int J Pharm Sci Res 2014; 5: 2490–07 doi:10.13040/IJPSR.0975-8232.5(6).2480-2497

[31] Xu Z, Wang X, Zhou M et al. The antidiabtic activity of total lignan from Fructusarctii against alloxan-induced diabetes in mice and rats. Phytother Res. 2008; 22: 97–101

[32] Hu FB, Li TY, Colditz GA et al. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. JAMA 2003; 289: 1785–1791