academicJournals

Vol. 5(3), pp. 19-28, April, 2014
DOI: 10.5897/JDE2014.0076
ISSN 2141-2685

http://www.academicjournals.org/JDE

Journal of Diabetes and Endocrinology

Full Length Research Paper

Protective effect of *Commiphora wightii* in metabolic activity of streptozotocin (STZ) induced diabetes in rats

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Received 18 February, 2014; Accepted 8 April, 2014

Herbal products are an affective move towards the treatment of diabetes and impeding its complications. However, there have been no reports to date on *Commiphora wightii* (EACWR) for the treatment of diabetes and its associated metabolism. Oral administration of EACWR (400 mg/kg body weight) to streptozotocin (STZ) induced diabetic rats effectively elevated body weight, plasma insulin level, hepatic and skeletal muscle glycogen and hepatic glucokinase (GK) activity. It also increases serum antioxidant levels (superoxide dismutase [SOD] and catalase [CAT]) and hepatic lipids like high density lipoprotein-cholesterol (HDL-C). Simultaneously, EACWR administration also lowers the plasma triglycerides (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), serum lipid peroxidation (LPO) and hepatic glucose-6-phosphatase (G-6-Pase) activity. It was also found that EACWR supplementation into diabetic rats also depleted inflammatory cytokines level (tumor necrosis factor-α [TNF-α], interlukin-6 [IL-6] and interlukin-1β [IL-1β]). This reduced cytokines level indicating the protective role of EACWR on pancreatic β-cell from apoptosis and promotes the exiting β-cell to release more insulin. Our significant outcomes suggest that EACWR has a potential impact in abrogating hyperlipidemia, oxidative stress and repaired the carbohydrate metabolism that could be considered as a protective herbal drug for diabetes treatment.

Key words: *Commiphora wightii,* diabetes, oxidative stress, carbohydrate, lipid metabolism.

INTRODUCTION

Diabetes mellitus (DM) is raised globally by increasing population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity. It is characterized by abnormal regulation of glucose and lipid metabolism (Jia et al., 2009). Diabetes mellitus is one of the major health issue in all the developed as well as developing countries, and the global incidence is projected to be double to 350 million till 2030 (Guilherme et al., 2008). Many drugs have been used for the prevention of diabetes, but due to some side effects such as flatulence and diarrhea, their formula needs to be improved or the new drugs modified. Therefore, the number of researchers and drug based companies are focusing their target to develop good agents from herbs or natural products (Chen et al., 2012).

Several studies have proved that, enormous production

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of reactive oxygen species (ROS) plays central role in the onset, development and pathological consequences of diabetes (Jakus, 2000). It also occurs as a result of impaired antioxidant defence system, that is, alteration in enzymatic and non enzymatic antioxidant level (Venkatesh et al., 2010). Hyperglycemia may evoke abundant free radicals via protein glycation and glucose autoxidation, which further catalyzes lipid peroxidation (Fridlyand and Philipson, 2005). Oxidative stress induces diabetic monocytes to liberate vast amount of superoxide anion (O_2^-) which in turn generates plasma and tissue lipid peroxidation products like malondialdehyde (MDA). Moreover, pancreatic β cells are largely prone to oxidative stress damage. There is pool of evidence that chronic hyperglycemia is the pivotal cause of retinopathy, neuropathies, renal malfunctions and cardiovascular complications in diabetes. Therefore, in hyperglycemic state, pancreatic β cells gradually worsen, and down regulates the secretion of insulin (Coskun et al., 2005). Insufficient insulin secretion leads to excessive lipolysis in the adipose tissue and results into hyperlipidemia. Diabetes is also caused by metabolic errors of carbohydrate, lipid and lipoprotein (McCune and Johns, 2002).

However, generation of oxidative stress also generates the development of inflammatory cytokines in the pancreas which is one of the major onsets of diabetes. Number of studies has been done on the role of inflammatory cytokines (tumor necrosis factor-α [TNF-α], interlukin-6 [IL-6] and interlukin-1β [IL-1β]) causing β-cells dysfunction and insulin resistance (Donath, 2013; Spranger et al., 2003); in order that the treatment of pro-inflammatory cytokines in the pathogenesis of type 2 diabetes opens the door for a causative treatment. Many impressive drugs have been developed for the treatment of many diseases by targeting these inflammatory cytokines, thus offers new opportunity to test numerous drugs against the pro inflammatory cytokines (TNF*α*, IL-1*β*, NF-*κ*B and IL-1*α*) in case of diabetes treatment.

For the past several years, natural products have received scientific and medicinal status with their antioxidant property which represents a better option for the treatment of diabetes (McCune and Johns, 2002). *Commiphora wightii* tree commonly known as guggul or oleoresin, belongs to the family of Burseraceae, a herbal resin. Earlier named as *Commiphora mukul*, it is used in Ayurveda as a medicinal-mineral herb to treat a variety of ailments, including obesity, arthritis, bone fractures, parasitic infection, gastrointes-tinal diseases, inflammation, cardiovascular diseases and lipid disorders centuries (Satyavati, 1988). Guggul has been traditionally used as anti-inflammatory, antispasmodic, antiseptic, carminative, astringent, thyroid stimulant, antihelminthic, hypoglycemic, and antihyperlipidemic properties (Shishodia et al., 2008). Various secondary metabolites including steroids, terpenoids, flavonoids, lignans, sugars, alcohol, etc., have been revealed in this genus (Zhu et al., 2001).

Antiproliferative, antimicrobial, hepatoprotective, anti-inflammatory, antidiabetic, cardiovascular and neuroprotective properties of the purified metabolites and the crude extracts have been explored (Deng, 2007; El Ashry et al., 2003; Ramesh and Saralakumari, 2012; Shen and Lou, 2008). Essential oil extract of guggul and guggulsterone were established as potent antioxidants against low density lipoprotein (LDL) oxidation (Siddiqui et al., 2013; Wang et al., 2002). Attenuating role of ethanolic extract of *C. mukul* has been illustrated in streptozotocin (STZ) induced oxidative stress in diabetic rats (Ramesh et al., 2012). The objective of the present study was to evaluate the ameliorative effects of ethyl acetate extract of *C. wightii* resin (EACWR-400 mg/kg body weight) on markers of glycemic control, oxidative stress, hyperlipidemia and its anti-inflammatory properties in STZ induced diabetes mellitus in Wistar albino rats.

MATERIALS AND METHODS

Plant extract preparation

Resins of *C. wightii* (Arn.) was collected locally from the botanical garden of Arawali hills of Jaipur in late October, 2012 and was identified by botanist Prof. Sandeep Kumar, Department of Botany, Nims University, Jaipur. The plant materials were taken to the laboratory and were authenticated. Washing of guggul resin was done with tap water, and sterilized with distilled water. They were further air-dried on filter paper at room temperature and then powdered with the pestle and mortar aseptically. Thorough mixing of air-dried powder (10 g) of resins with ethyl acetate solvent (100 ml) was done for 24 h on orbital shaker (150 rpm) at room temperature. Solution was filtered through muslin cloth and then re-filtered through Whattman's Filter No. 1. The filtrate was concentrated by complete evaporation of solvent at 25°C to yield the pure extracts. Stock solutions of *C. wightii* extracts and solvent were prepared in 100 mg/ml concentration. Then, the solution was stored in refrigerator in sterilized bottles until further used.

Animals

Thirty-two male albino Wistar rats (250 to 300 g) were used in this study. The animals were housed in polypropylene cages separately under standard conditions (12 h light and dark cycles, at 25±30°C and 35 to 60% humidity). All animals were allowed free access to the same standardized diet and water during the entire study. The study was approved by Institutional Animal Ethical Committee, NIMS University, Jaipur, Rajasthan, India.

Experimental animal models and design

The experiment was done by following the single intraperitoneal injection of fresh STZ solution (40 mg/kg/body weight, in ice cold 0.1 M citrate buffer, pH 4.5 in a volume of 0.1 ml/rat) prepared to induce diabetes in overnight fasted rats. After 72 h of STZ induction, blood was collected from rat tail fasted 12-h in fresh vials containing ethylenediaminetetraacetic acid (EDTA 10 mg/ml) for fasting blood glucose measurements. Fasting blood glucose was measured by glucose oxidase method using blood sugar meter and blood sugar test paper (Johnson & Johnson, USA). Rats with fasting

fasting blood glucose of ≥11.1 mmol/l were considered as diabetic. Insulin was determined by radioimmunoassay using rat insulin as standard (Linco Research, St. Charles, MO, USA). The estimation of hemoglobin (Hb) was done by using method of Drabkin and Austin (1932). Glycosylated serum protein was measured by fructose amine nitrogen blue four triazole method. The control and diabetic rats were then divided into 4 groups of 8 each: (1) control (C) group (rats treated with saline in a matched volume); (2) control rats treated with EACWR (C+EACWR); (3) diabetic model group (D) (diabetic rats treated with saline in a matched volume); and (4) diabetic rats treated with EACWR (D+ EACWR). Diabetic and control + EACWR groups received 400 mg/kg body weight of an EACWR by orogastric tube for 30 days. At the end of the experimental period, the animals were fasted overnight and sacrificed by anaesthetic overdose of sodium pentabarbitol (40 mg/kg). The body was cut open and liver was dissected out and thoroughly rinsed with ice cold saline.

Lipid profile of guggul treated versus control

The liver homogenate was subjected to evaluate lipid profile such as total cholesterol (TC) (Wybenga et al., 1970), triglycerides (TG) (McGowan et al., 1983), high density lipoprotein-cholesterol (HDL-C) (Burstein et al., 1970), LDL-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) = Total Serum Triglycerides/5 LDL-C = Total serum cholesterol - total serum triglycerides- HDL-C/5.

Study of oxidative stress markers

Serum was used to evaluate oxidative stress markers, namely, catalase (EC 1.11.1.6) was estimated according to the method of Aebi (1974). For superoxide dismutase (EC 1.15.1.1), samples were treated, and superoxide dismutase (SOD) activity was determined by the method described earlier by McCord and Fridovich (1969) and lipid peroxidation by Ohkawa et al. (1979).

Determination of insulin

Serum insulin was quantified by Glazyme Insulin-EIA test from diabetic rats. 400 mg/kg body weight of EACWR were orally administered to the rats for 30 days and blood samples were taken for insulin determination. The insulin content in serum was expressed in µIU/ml.

Assay of glycogen content

Liver and skeletal muscle of rats was removed. Liver tissues (100 mg) were homogenized in 5 volumes of an ice-cold 30% (w/v) KOH solution and were dissolved in a water-bath at 100^{o}C for 30 min. Precipitation of glycogen in the ethanol, was washed, and resolubilized in distilled water. Thereafter, amount of glycogen was assayed by the anthrone reagent method (Seifter et al., 1950). The amount of blue compound generated was determined by a spectrophotometer at 620 nm. The glycogen content was expressed as mg/g wet tissue.

Assay of glucose-6-phosphatase and protein

Glucose-6-Phosphatase (G6Pase) activity was assayed by the method of Trinder (1969). In liver, the glucose-6-phosphate was converted into glucose and inorganic phosphate. The inorganic phosphate liberated was evaluated with ammonium molybdite and ascorbic acid was used as the reducing agent. Surplus molybdate was removed by the arsenite citrate reagent, so that it could not react with other phosphate esters or with inorganic phosphate formed by acid hydrolysis of the substrate. The phosphate content was determined as blue phosphor-molybdenum complex at 700 nm by spectrophotometer; it shows the glucose-6-phosphatase activity. Protein level of liver homogenate was quantified with Bradford reagent (Bradford, 1976). G-6-Pase activity (mU) was expressed as mmol of phosphate released/min/mg of protein.

Assay of glucokinase activities in liver

Glucokinase (GK) activity was evaluated using a method illustrated by Panserat et al. (2001). β-nicotinamide-adenine dinucleotide phosphate (NADPH) generated by GK was measured spectrophotometrically at 340 nm. GK activity was estimated by subtracting the rate of NADPH formation in the presence of 0.5 mM glucose from that obtained in the presence of 100 mM glucose. Protein level was quantified with Bradford reagent and one unit of enzyme activity (mU) was defined as mmol of substrate molecules converted by 1 mg protein/min.

Cytokines study

After every 15 days, blood samples were collected by tail vein in tubes containing EDTA (10 mg/ml). The sample was separated by centrifugation at 3000 rpm for 5 min and the plasma obtained was aliquoted and frozen for insulin estimation and for the analysis of serum cytokines levels (IL-6, IL-1β and TNF-α). The cytokines assay was determined using the quantitative sandwich enzyme immunoassay technique (Invitrogen). A monoclonal antibody specific for rat IL-6, IL-1β and TNF-α were pre-coated onto a micro plate. Standards, controls and samples were pipetted into the wells and rat IL-6, IL-1β and TNF-α that present in the sample is bound to the immobilized antibody. After washing, unbound substances were washed out, an enzyme-linked polyclonal antibody specific for rat IL-6, IL-1β and TNF-α was then added to the wells. Washing was done to remove any unbound antibody-enzyme reagent, after which substrate solution was added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution was added. The intensity of the color measured is in proportion to the amount of rat IL-6, IL-1β and TNF-α bound in the initial step. The sample values are then read off the standard curve.

Statistical analysis

Results are expressed as mean±standard error mean (SEM) and subjected to one-way analysis of variance (ANOVA), followed by Student Newman-Keuls post hoc test and values with $p<0.05$ were considered to be statistically significant. InStat (version 3) was used for analysis of data.

RESULTS

Effect of EACWR on body weight

The body weight of all the animals was measured at different time intervals. STZ induced D group of animals showed gradual loss of body weight during the experiment as compared to the control group. Significant increase in body weight was observed on orally administrated D+EACWR groups as compared to STZ induced in

group D at different time interval during the experimental duration (Figure 1). On the other hand, C+EACWR treated group exhibited insignificant changes in the body weight ($p>0.05$) as compared to group C.

Effect of EACWR on fasting glucose level, Hb and glycosylated serum protein

Figure 2a, b and c represents the fasting glucose level, Hb and glycosylated serum protein both control and experimental rats at time interval of 07, 15 and 30 days period of study. Decreased Hb levels significantly ($p<0.05$) increased D+EACWR treated group and these values come back near to normal level. The fasting glucose and glycosylated serum protein level in D+EACWR was found to be decreased as compared to diabetes group ($p<0.01$).

Plasma insulin study

Fasting plasma insulin level was estimated at the end of experiment in all the four groups of animal (Figure 3). D group showed decreased ($p<0.001$) plasma insulin level as compared to C group. D+EACWR group of rats have demonstrated a significantly higher insulin concentration when compared with D group after 30 days of EACWR administration. The insulin level of D+EACWR group was significantly elevated than D group ($p<0.01$). Plasma insulin level was found to increase significantly in the C+EACWR group as compared to the control treated group ($p<0.01$) and hence increased insulin level by EACWR administration and maintains the blood glucose level.

Effect of EACWR on oxidative stress status in serum

Table 1 reveals the activities of enzymatic antioxidants (SOD and CAT) in serum of C, D, C+ EACWR and D+ EACWR rats. SOD and CAT decreased significantly ($p<0.001$) in D group of rats. Administration of EACWR significantly improved the activities of SOD ($p<0.05$) and CAT ($p<0.001$) enzymes in D+EACWR treated group when compared with D rats. Lipid peroxidation rate were increased ($p<0.001$) in D group rats as compared to C group, whereas suppression of MDA products were observed in D+EACWR ($p<0.001$) and C+EACWR treated groups ($p<0.001$).

Effect of EACWR on hyperlipidemia in liver

D group of rats showed significant increase in liver total cholesterol, triglycerides ($p<0.01$), LDL cholesterol, and VLDL-cholesterol ($p<0.001$), whereas there was a significant decrease in HDL cholesterol ($p<0.001$) as compared to C group of animals. C+EACWR group resulted in significant decrease in liver TC ($p<0.05$), TG ($p<0.05$), LDL-C ($p<0.001$), VLDL-C ($p<0.001$) and non-significant increase in HDL-cholesterol ($p>0.05$) in comparison to C group. D+EACWR group showed a significant decline in liver TC ($p<0.05$), TG ($p<0.05$), LDL-C ($p<0.001$), VLDL-C ($p<0.001$) and a significant increment in HDL-C ($p<0.01$) when compared with D group of rats (Table 2).

Effect of EACWR on carbohydrate metabolism

The results of the present study revealed that there is an increase ($p<0.001$) in the glycogen content of liver and skeletal muscle of D+EACWR rats as compared to group D (Table 3). Glycogen level was significantly declined ($p<0.001$) in group D of liver and skeletal muscle as compared to group C. G6Pase activity was significantly decreased ($p<0.001$) in D+EACWR rats as compared to group D, whereas it was significantly increased ($p<0.001$) in group D as compared to group C. GK activity was increased ($p<0.001$) in D+EACWR group as compared to group D. EACWR declined G6Pase action and increased glycogen and GK activity in liver, which illustrates increased hepatic glucose uptake and decreased hepatic glucose release. So, EACWR facilitate hypoglycaemic activity.

Effect of EACWR on serum cytokines level

The serum cytokines level e.g. TNF-α, IL-6 and IL-1β were recorded at every 15 days of time interval. The significantly increased ($p<0.001$) level of TNF-α, IL-6 and IL-1β were found in group D rats as compared to C and C+ EACWR treated control groups. EACWR supplementation were decreased the serum TNF-α, IL-6 and IL-1β significantly ($p<0.01$) in D+EACWR treated group as compared to D group of rats and the values was almost near to the control group (Figure 4a, b and c).

DISCUSSION

Diabetes is a metabolic disorder caused by the insufficient supply of insulin, a hormone that is required to convert sugar, starches and other food into energy in the human body. The conventional therapies for the treatment of diabetes have many side effects and high rate secondary failure. The present study was carried out to investigate the efficacy of EACWR as an anti-diabetic drug on STZ induced diabetic rats. There are two main of hypothesis of STZ employed for the stimulation of diabetes mellitus in experimental rats (Kavalali et al., 2003) like disarrangement of pancreatic β-cells in islets of Langerhans of the pancreas (Tomlinson et al., 1992).

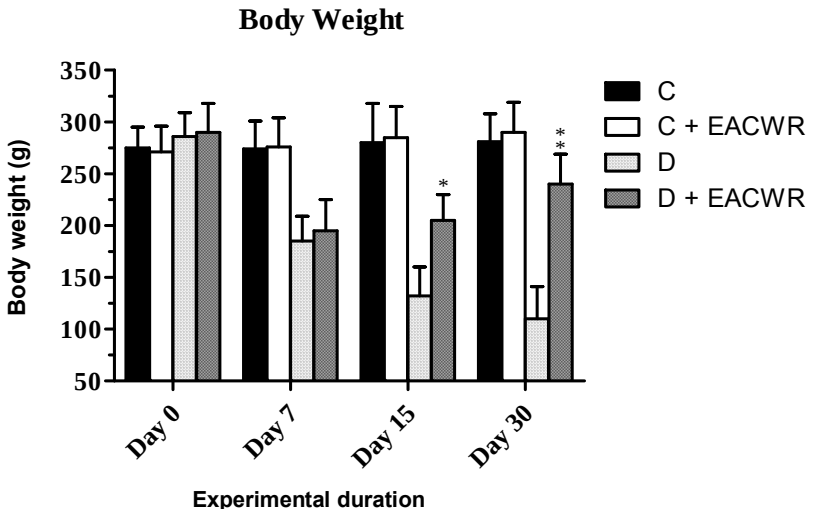

Figure 1. Effect of EACWR on body weight of control and STZ induced diabetes rats. Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D and (b) group D vs. D+EACWR, and (c) group C vs. C+EACWR. **p<0.01 significant and *p<0.05 significant. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

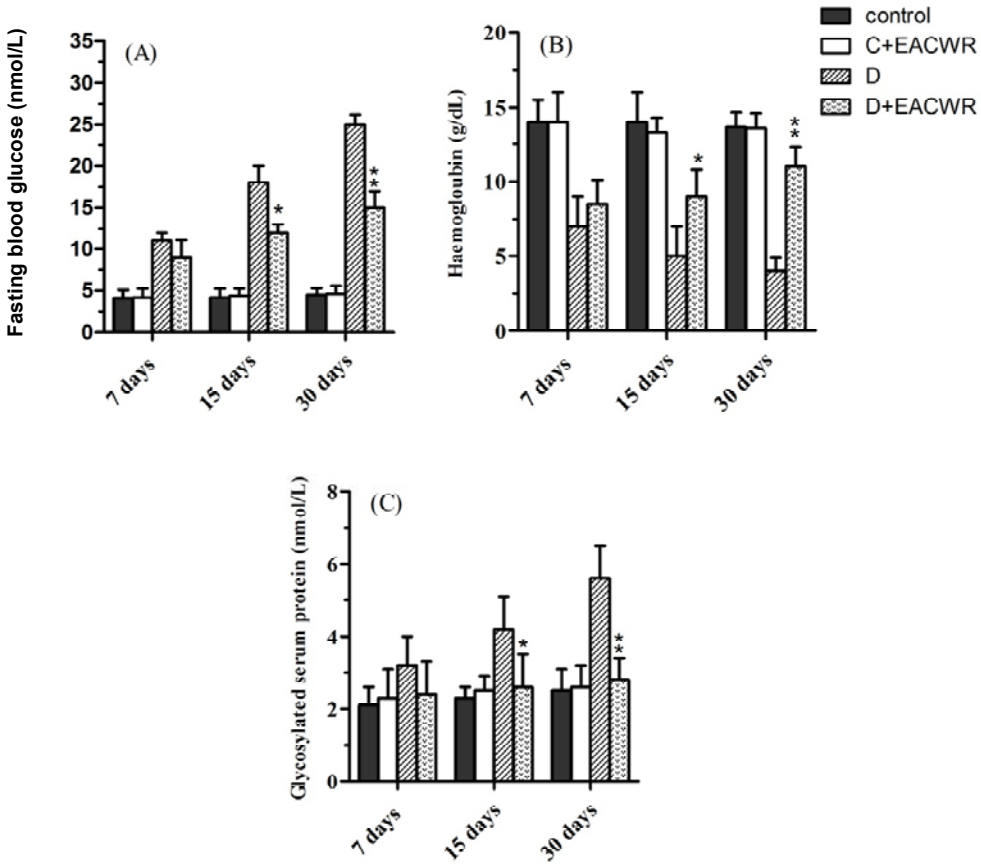

Figure 2. Effect of EACWR on fasting blood glucose, haemoglobin and glycosylated serum protein at different time intervals of control and STZ induced diabetes rats. Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR. **p<0.01 and *p<0.05 significant. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

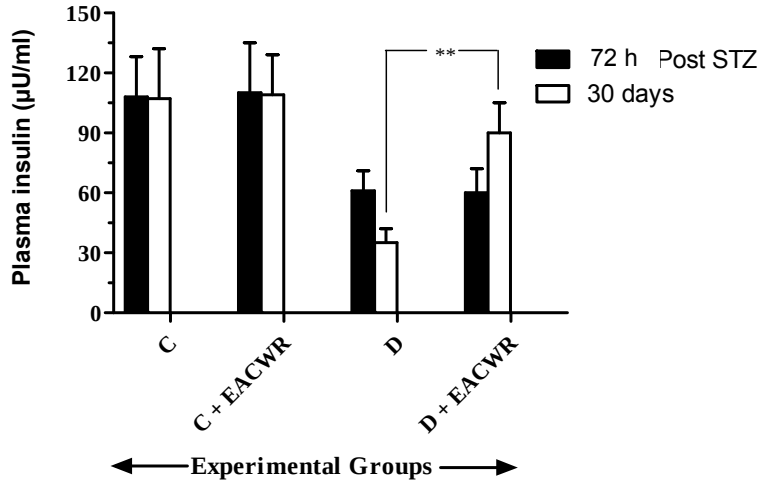

Figure 3. Effect of EACWR on plasma insulin level in serum of control and STZ induced diabetes rats. Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR. **p<0.01 and *p<0.05 significant. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

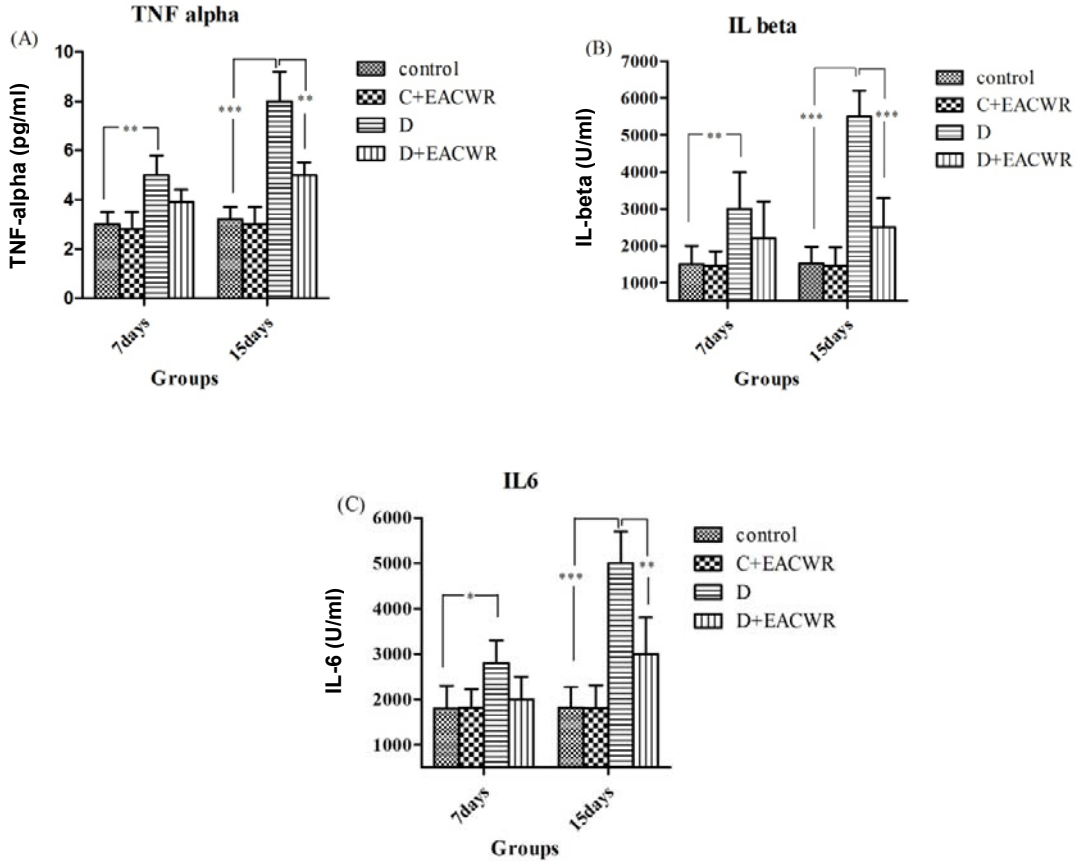

Figure 4. Effect of EACWR on serum cytokines level of control and STZ induced diabetes rats. Values are expressed as Mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR . ***p<0.001 highly significant, **p<0.01 significant and *p<0.05 significant. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

Table 1. Effect of EACWR on oxidative stress in serum of control and STZ induced diabetic rats.

Parameter	C	C+EACWR	D	D+EACWR
SOD	47.67±1.23	50.81±0.29[c]	36.68±1.39[a]	40.16±1.24[b]
CAT	95.70±1.93	107.17±1.92[c]	74.32±1.91[a]	86.30±1.95[b]
LPO	21.31±0.32	19.72±0.55[c]	34.02±0.60[a]	26.15±0.27[b]

Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR. Superscript symbols represent statistical significance: $p<0.05$. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

Table 2. Effect of EACWR on lipid profile in liver of control and STZ induced diabetic rats.

Parameter	C	C+EACWR	D	D+EACWR
TC (mg/dl)	70.55±2.05	64.30±3.58	84.13±2.33[a]	76.96±5.07[b]
TG (mg/dl)	74.43±3.31	63.21±2.28[c]	92.14±3.09[a]	80.90±3.39[b]
LDL (mg/dl)	26.61±0.42	19.08±0.26[c]	39.23±0.32[a]	30.57±0.28[b]
VLDL (mg/dl)	13.21±0.21	10.28±0.27[c]	21.08±0.51[a]	16.62±0.39[b]
HDL (mg/dl)	25.63±0.19	27.01±1.59	14.45±2.02[a]	20.64±1.64[b]

Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR. Superscript symbols represent statistical significance: $p<0.05$. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

Table 3. Effect of EACWR on carbohydrate metabolism in control and STZ induced diabetes rats.

Parameter	C	C+EACWR	D	D+EACWR
Glycogen (in liver)	15.18±0.18	12.16±0.41[c]	9.22±1.06[a]	17.48±0.07[b]
Glycogen (in skeletal muscle)	13.60±0.19	11.73±0.13	6.42±0.64[a]	10.04±1.26[b]
GK (in liver)	3.41±0.08	3.74±0.04[c]	1.56±0.04[a]	2.37±0.0[b]
G-6-Pase (in liver)	0.35±0.01	0.32±0.04[c]	0.77±0.02[a]	0.56±0.05[b]

Values are expressed as mean±SEM, n=8 animals in each group. Comparisons were made between: (a) group C vs. D; (b) group D vs. D+EACWR; (c) group C vs. C+EACWR. Superscript symbols represent statistical significance: $p<0.05$. STZ: Streptozotocin; EACWR: ethyl acetate extract of *C. wightii* resin; SEM: standard error of mean.

Secondly, STZ deteriorate the structure of DNA in pancreatic islets and stimulating nuclear poly (ADP-ribose) synthetase, and thus suppressing intracellular NAD^+ levels, which later inhibits proinsulin synthesis and induces diabetes (Wilson et al., 1988). The intraperitoneal administration of single low dose of STZ (40 mg/kg) selectively destroys some population of pancreatic beta cells resulting in insulin deficiency and causing type 2 diabetes (Balamurugan et al., 2011; Frode and Medeiros, 2008). In diabetes mellitus, insulin is not or insufficiently synthesized, developing hyperglycemia with biochemical changes in glucose metabolism.

This study demonstrated that EACWR prohibited the elevation in fasting blood glucose, haemoglobin, glycosylated serum protein, and fasting plasma insulin, which were all increased in the diabetic rats. Thus, the *in vivo* studies suggest that EACWR may be beneficial for prevention and treatment of diabetes. EACWR might be supporting to existing β-cells of islets of Langerhans to release more insulin and lowering the increased glucose. This lowering of blood glucose level by EACWR supplementation might be due to pancreatic secretion of insulin from regenerated β-cells, or its action to release bound insulin from regenerated β-cells by inhibiting ATP sensitive K+ channels (Insulin secretion from pancreatic islets study is under progress in our laboratory). Studies suggest that normal plasma insulin is essential to maintain the glucose homeostasis by enhancing the glycolysis and glycogen synthesis in skeletal muscle, with the concomitant decrease in glycogenolysis in liver and skeletal muscles (Shimazu, 1987).

Moreover, significant loss of body weight, enhanced muscle and tissue proteins wasting in STZ induced diabetic rodent models due to the altered carbohydrate metabolism (Raju et al., 2001; Swanston-Flatt et al., 1990). EACWR supplementation raised the body weight of diabetic rats significantly near to control. This may be due to the defensive effect of the EACWR in muscle and protein wasting as well as improvement in the rate of insulin secretion and carbohydrate metabolism.

Hepatic lipid profile is generally high in case of diabetes mellitus and such an elevation predicts risk factor for cardiovascular disease. TC, TG, VLDL and LDL were remarkably increased and HDL cholesterol was decreased

decreased in group D of rats. There was a significant downfall in TC, LDL-C, VLDL-C, TG and rise in HDL-C level of D+EACWR treated group and close to C group. It may be interpreted from the data that EACWR may be linked with enhanced insulin secretion (Nityanand and Kapoor, 1973). The anti-diabetic and anti-lipidemic action of EACWR may be attributed to the presence of plant sterols like guggulsterone-E and guggulsterone-Z (Urizar et al., 2002).

High rate of LPO is ascribed to the increased liberation of reactive oxygen species (ROS). Reactive carbonyls compounds are the oxidative stress marker and could contribute to pathogenesis of diabetes (Taleb-Senouci et al., 2009) (Table 1). In the present study, MDA, a LPO product formation was significantly increased in serum of STZ treated diabetic animals. EACWR administration has suppressed the MDA level suggesting that EACWR might have profound antioxidant response. Impaired membrane functions, inactivation of membrane bound enzymes and receptors are consequent effects of LPO (Chen et al., 2002).

Diabetes mellitus is also associated with oxidative stress, that is, imbalance between free radicals production and antioxidant level (Naziroglu and Butterworth, 2005). Oxidative stress resulted in altered activity of antioxidant enzymes, such as SOD and CAT (Maritim et al., 2003). The present findings of EACWR supplementation have restored SOD and CAT activity demonstrated by enhanced free radical scavenging activity which could abrogate pathological alterations in group D rats. Benhamou et al. (1998) stated that expression of CAT in islets cell had minimized the H_2O_2 induced deformities and declined the progression of STZ induced diabetes in mouse.

To the best of our knowledge, this is the first study to evaluate the effect of EACWR on inflammatory cytokines on STZ induced diabetic rats. EACWR supplementation reduces the effect of increased level of TNF-α, IL-6 and IL-1β in the diabetic rats. There are several investigations that have been done into the role of inflammatory cytokines in the progression of diabetes (King, 2008; Spranger et al., 2003). A mechanism has been proposed linking the expression of TNF-α and other inflammatory mediators to the development of insulin resistance in obesity and type 2 diabetes (Huang et al., 2009; Leiherer et al., 2013; Petersen et al., 2004). Under normal physiologic conditions, insulin interacts with insulin receptors to stimulate two main pathways in cardiovascular tissues: a phosphoinositide-3 kinase (PI3K) pathway that inhibits atherogenesis and has antiatherogenic effects and a mitogen-activated protein kinase (MAPK)-activated pathway that promotes cellular growth and enhances atherogenesis (Das Evcimen and King, 2007; Scott and King, 2004). In the presence of insulin resistance and diabetes, the increase in glucose and free fatty acids leads to an increased release of inflammatory cytokines (TNF-α, IL-6 and IL-1β) and altered regulation of protein kinase C (PKC) and MAPK activity. (Das Evcimen and King, 2007; Scott and King, 2004). PKC inhibits the PI3K pathway and results in enhanced atherogenesis through such processes as a reduction in antiatherogenic nitric oxide production and impaired endothelium-dependent vasodilation. In addition, processes initiated by insulin resistance and diabetes stimulate the MAPK pathway to exert proatherogenic actions. The mechanism of EACWR for the inhibition of these increased inflammatory cytokines is under investigation in our laboratory. According to the present results, supplementation of EACWR significantly reduces the inflammatory effect of TNF-α, IL-6 and IL-1β in diabetic rat models and shows its potential effect does not minimize cytokines levels.

In addition, the attenuating effect of *C. wightii* extract on experimentally induced diabetes has been established here by estimating the glucose-6-phosphatase, glucokinase activity in liver, and glycogen in liver and skeletal muscle, which is the predictor of the diabetes mellitus. EACWR supplementation increases hepatic glycogen content and skeletal muscle on D+EACWR treated group as compared to group D. G6Pase activity was also suppressed in D+EACWR treated group of experimental animals as compared to group D (Table 3). GK activity was found to be increased by EACWR in D group of rats. These effects may be obtained as a result of suppressed rate of cholesterol biosynthesis and or declined level of lipolysis which are influenced by insulin (Boby and Leelamma, 2003). There are various probable mechanism of hypoglycemic activity of EACWR that may be regulated by the serum insulin assay in diabetes rats. EACWR may be crucial in the sensitivity of insulin receptor in target organ and is helpful to maintain the blood glucose level in STZ diabetic rats.

Finally, it was concluded that EACWR have attenuating effects on hyperlipidemia, hypoinsulinemia, anti-inflammatory and oxidative stress in STZ induced diabetic rats. It is well versed in improving carbohydrate metabolism. Moreover, the further exploration of major phytoconstituents of *C. wightii* is needed to improve its activity to enhance its medicinal importance. Thus, the present findings provide a vast evidence for the therapeutic potential of EACWR that might be considered as a safe and new drug candidate for the treatment of DM.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Sandeep Kumar, Professor and Head, Department of Botany, NIMS University, Jaipur India for identifying and suggesting *Commiphora wightii* as a novel Indian medicinal drug and provide phytochemical analysis details of the plant; and also to the NIMS University administration for their kind financial support.

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