PROTECTIVE ROLE OF *COCCINIA INDICA* HERB AGAINST DEXAMETHASONE-INDUCED INSULIN RESISTANCE

Pharmacology

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ABSTRACT

Introduction: *Coccinia indica* is widely used medicinal herb in Ayurveda. The objective of this study was to explore the anti-diabetic and hypolipidemic activity of *Coccinia indica* in dexamethasone induced insulin resistance.

Methods: Male *albino* rats were divided in to 5 groups (n=6). Group 1(control) rats received 2% gum acacia orally. Group 2(Diabetic control) rats were administered 2% gum acacia orally for 12 days and dexamethasone (8 mg/kg/i.p.) from 7th to 12th day. Group 3(Test group) and Group 4(Standard control) animals were treated with aqueous extract of *Coccinia indica* leaves (2 gm/kg) and metformin (2 gm/kg) respectively. All groups except control rats were treated with dexamethasone (8mg/kg/i.p.) from 7th to 12th day. Fasting blood was collected and subjected to biochemical investigations.

Results: Pre-administration of aqueous extract of *Coccinia indica* leaves(2gm/kg) significantly (p<0.05) prevented the elevation of insulin, glucose and lipid levels in dexamethasone induced insulin resistant rats.

Conclusion: It was concluded that the aqueous extract of *Coccinia indica* leaves can be useful in the prevention of glucocorticoid induced diabetes.

KEYWORDS:

Hyperlipidemia, Insulin sensitizer, Glucocorticoids, Metformin

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the frequent form of diabetes. Insulin resistance (IR) is a major component of T2DM which has been in epidemic proportions.[1] IR is known as decreased sensitivity of peripheral tissues towards insulin action.[2] It is often accompanied with complications like T2DM, cardiovascular abnormalities, dyslipidemia, hypertension and central obesity etc.[3]

Elevated free fatty acid levels have ability to reduce the glucose transport into muscle by impairing the insulin signaling leads to development of IR.[4] Hence, it is beneficial to detect the IR in early stages to prevent the future risk of metabolic syndrome. World Health Organization (WHO) has opined that there is a need to evaluate the efficacy of traditional plants and to develop effective interventions for T2DM.[5]

Coccinia indica (*C.indica*) belongs to Cucurbitaceae family and frequently employed in traditional Indian medicine.[6] The leaf of *C.indica* plant possess anti-hyperglycemic, antioxidant, hypolipidemic and anti-inflammatory activity.[7,8] But, none of the studies explained its insulin sensitizing property. Therefore, the present study focused on protective role of aqueous extract of *C.indica* leaf against dexamethasone (DEX) induced IR in rats.

MATERIALS AND METHODS
Experimental animals
Male albino rats of the Wistar strain weighing around 250-300gm were used and accommodated in polypropylene cages (UN shah manufacturers, Mumbai). The permission was obtained from the Institutional Animal Ethics Committee (KSHEMA/IAEC/02/2013). As per the regulations of Committee for Purpose of Control and Supervision on Experimental Animals (CPCSEA) rats were maintained under standard conditions, 23±2°c temperature, humidity 50±5%, 12:12 hrs light and dark cycles. They had free access to pellet food (Hindustan lever limited, Mumbai) and water.

Drugs and chemicals
Metformin (MET) drug was obtained from Mahalakshmi chemicals, Hyderabad. DEX drug was procured from Zydus pharmaceuticals, Mumbai. Ketamine injection was purchased from Neom Laboratories Limited, Mumbai. Serum insulin levels were assessed by using ultrasensitive rat insulin Enzyme-Linked Immuno Sorbent Assay (ELISA) kit, purchased from the Gen X Bio Health Sciences Private Limited, New Delhi. Glucose and lipid reagent kits were purchased from Agappe Diagnostic Limited, Bangalore.

Identification and preparation of aqueous extract of *C.indica* leaves
Around 4kg of *C.indica* leaves was collected from local garden, Nalgonda, Telangana and authenticated by Dr.Sunil kumar KN, Senior Research officer, Department of Pharmacognosy, SDM centre for research in Ayurveda and Allied sciences, Udupi, Karnataka.

The dried leaf material was suspended with 18 liters of ethanol was added into a round bottom flask and made it to stand for 24 hrs. Further, the contents were filtered and concentrated by distillation. In the end, the extract was subjected to water bath to remove solvent and completely dried under vacuum. Finally, 125.77gm of yield was obtained and stored for further use.[8]

Study protocol
The study was conducted for a period 12 days. Male albino rats were randomized in to 4 groups (n=6). Group 1 (normal control) rats received 2% gum acacia (10ml/kg/oral) for 12 days. Group 2 (diabetic control) rats were treated with 2% gum acacia for 12 days and intraperitoneal (i.p.) DEX (8mg/kg) from 7th to 12th day. Group 3(test group) rats received oral administration of aqueous extract of *C.indica* (2 gm/kg) and Group 4(standard control) animals were treated with MET (2 gm/kg) orally for 12 days. All groups except normal control treated with DEX (8mg/kg/i.p.) from 7th day to 12th day. On last day, fasting blood was collected through retro-orbital sinus puncture under ketamine (50mg/kg/i.p.) anaesthesia. The blood samples were centrifuged at 2000RPM for 20 minutes and serum used for estimation of glucose, insulin and lipid levels.

Measurement of Biochemical parameters:
Serum glucose levels were estimated by glucose oxidase and peroxidase (GOD-POD) method. Serum Insulin levels were assessed by Enzyme-Linked Immunosorbent Assay (ELISA) method. Serum triglyceride levels were investigated by using Glycerol 3-phosphate oxidase phenol aminophenazine (GPO-PAP) method. Total cholesterol, HDL-C and LDL-C levels were estimated by Cholesterol oxidase phenol aminophenazine (CHOD-PAP) method.[9]

Phytochemical analysis

Aqueous extract of *C.indica* was tested for the presence of phytochemical constituents such as alkaloids, steroids, carbohydrates, tannins, flavonoids, saponins, terpenoids, coumarins, phenols, carboxylic acids, resins, quinones and amino acids by standard tests.[10]

Statistical analysis:

The values were assessed by using one-way ANOVA followed by Scheffe's multiple comparison post hoc test. The level of significance was set at $p < 0.05$.

RESULTS
Biochemical parameters
Table 1: Effect of aqueous extract of *C.indica* leaves on serum glucose and insulin levels against DEX induced IR

Groups	Serum Glucose (mg/dl)	Serum Insulin (ng/ml)
Group 1	101.18±0.97	2.81±0.09
Group 2	268.23±2.13[*acd]	18.27±0.36[*acd]
Group 3	133.79±5.96[*b]	7.23±0.48[*b]
Group 4	126.10±2.36[*b]	6.59±0.30[*b]

Values are expressed as mean±SEM; *The mean difference is significant at the $p < 0.05$; Group 1- normal control[a]; Group 2-diabetic control[b]; Group 3 - aqueous extract of *C.indica* leaves(2gm/kg) [c]; Group 4 – Metformin(2gm/kg)[d]

Serum insulin and glucose levels: As shown in Table 1, DEX administration significantly ($p < 0.05$) elevated serum glucose and insulin levels in DEX treated group compared to control. Administration of aqueous extract of *C.indica* leaves (2gm/kg) significantly ($p < 0.05$) lowered the elevation of serum glucose and insulin levels after 12 days of treatment compared to diabetic control group. MET also significantly ($p < 0.05$) lowered the fasting glucose and insulin levels compared to diabetic control group (Table 1).

Table 2: Effect of aqueous extract of *C.indica* leaves on serum lipid levels against DEX induced IR

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C(mg/dl)	HDL-C(mg/dl)
Group 1	75.16±1.92	50.77±2.52	13.99±0.51	25.89±0.67
Group 2	216.98±2.45* acd	185.22±2.37* acd	104.49±1.81* acd	10.54±0.37* acd
Group 3	99.67±2.84[*b]	89.05±3.14[*b]	60.17±1.49[*b]	21.59±1.32[*b]
Group 4	82.50±2.07[*b]	73.02±2.37[*b]	46.69±1.55[*b]	21.20±0.45[*b]

Values are expressed as mean±SEM; *The mean difference is significant at the $p < 0.05$; Group 1- normal control[a]; Group 2-diabetic control[b]; Group 3 - aqueous extract of *C.indica* leaves(2gm/kg) [c]; Group 4 – Metformin(2gm/kg)[d]

Serum lipid levels: As shown in Table 2, DEX treatment significantly ($p < 0.05$) increased total cholesterol, triglycerides, LDL-C and decreased HDL-C levels. Pre-treatment with ethanolic extract of *C.indica* (2gm/kg/oral) and MET (2gm/kg/oral) had significantly ($p < 0.05$) inhibited the elevation of total cholesterol, triglycerides, LDL-C, and increased the HDL-C levels in diabetic rats (Table 2).

Phytochemical analysis
Table 3: Phytochemical constituents of *C.indica* extract

Test	Present(+)/Absent(-)
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Resins	+
Phenols	+
Triterpenoids	+
Steroids	-
Coumarins	-
Carboxylic acids	-
Quinones	-
Saponins	+
Tannins	+
Aminoacids	-

DISCUSSION

IR plays a crucial role in the development of metabolic syndrome.[11] Our study with DEX showed a significant elevation in serum glucose and insulin levels in Wistar rats. Previous studies have suggested that a high serum insulin concentration in response to increased glucose concentration suggests a state of IR.[12,13] In addition, DEX treatment also increased total cholesterol, triglycerides, and LDL-C levels and reduced HDL-C levels in rats. The probable mechanism of DEX induced IR likely to be due to impairment of insulin signaling/action and inhibits translocation of GLUT4 to plasma membrane.[14] Furthermore it elevates lipoprotein lipase function in adipose tissue contributes to reduced insulin sensitivity.[15]

In the present study, administration of aqueous extract of *C.indica* significantly prevented DEX-induced raise in serum glucose and insulin levels. Antidiabetic activity of aqueous extract of *C.indica* might be attributed to the presence of various phytochemical constituents i.e. triterpenes, flavonoids and saponins etc. found to have antidiabetic activity.[16] The plant extract may act by depressing key gluconeogenic enzymes i.e. glucose-6-phosphatase and fructose 1,6-biphosphatase.[17]

Pre-treatment with aqueous extract of *C.indica* significantly prevented the lipid levels in DEX treated rats. The glucose lowering activity of plant extract may contribute to hypolipidemic activity.[18] It was thought to be the triterpenoids which stimulate peroxisome proliferator activated receptor-ϒ and sensitizes the tissues to insulin contributes to insulin sensitizing and antidiabetic activity.[19,20] Treatment with *C.indica* leaf extract was equally efficacious to standard drug MET (2gm/kg). This study established the new insight that the *C.indica* possesses insulin sensitizing property in addition to insulin stimulating activity in glucocorticoid induced IR.

CONCLUSION:

Treatment with aqueous extract of *C.indica* leaves can produce insulin sensitizing and hypolipidemic activity which would be beneficial in the treatment of T2DM. Further research is required to establish the underlying mechanism of insulin sensitizing action of *C.indica*.

CONFLICT OF INTEREST: Nil

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