Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

An Investigation of Type II Anti-diabetic Activity of Biologically Active Phytochemical(s) of *Coccinia indica* Fruits in High Sugar Diet-Induced Diabetic Rats

Abdullah A. M. Ali, U. Rajashekhar, Deepak K. Jha*

Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka, India.

ARTICLE INFO

Article history:

Received: 19 October, 2023

Revised: 21 December, 2023

Accepted: 30 December, 2023

Published: 30 January, 2024

Keywords:

Anti-diabetic activity, *Coccinia indica,* Insulin, Anti-oxidant enzymes, Pro-inflammatory cytokines.

DOI:

10.25004/IJPSDR.2024.160108

ABSTRACT

Unhealthy diets and lifestyles cause a resistance to and/or relative deficiency of insulin production. Therefore, it is thought worthwhile to develop a natural remedy that may effectively manage the disease symptoms to a certain extent without causing adverse consequences. The objective was to develop the active biological constituent(s) for the use of *Coccinia indica* and its relationship to treating type II diabetic rats. The soxhlet extraction method was used to get the cocktail of phytochemicals of *C. indica* by using methanol. The composition of a high-sugar diet, followed by fructose (66%), was used to induce T2DM in rats. The preliminary predictive markers were body weight (pre- and post-treatment), blood glucose level (pre- and post-treatment), serum insulin, and pancreatic insulin. And the secondary outcomes were the pro-inflammatory mediators interleukin 6 (IL-6), transforming growth factor-β (TGF-β), and tumour necrosis factor alpha (TNF alpha). Additionally, pancreatic tissue was used to estimate beta cell mass, size, and necrosis, and the cell supernatant was used to observe superoxide dismutase (SOD), lipid peroxidation (LPO), and catalase (CAT). High sugar diet showed significant increase in body weight ($p < 0.01$), fasting blood glucose level ($p < 0.001$), and decrease in serum and pancreatic insulin levels ($p < 0.001$), whereas rats treated with methanolic extract of *C. indica* showed significant reduction in post-treatment body weight ($p < 0.01$), blood glucose levels ($p < 0.01$), and increase in serum and pancreatic insulin ($p < 0.001$), especially in higher doses, i.e., 400 mg/kg. Pro-inflammatory cytokines (IL-6, TGF-beta, and TNF-α) can increase insulin resistance, which results in poor glucose homeostasis, which has been reduced by treatment with *C. indica* ($p<0.001$). Superoxide radicals and a deficiency in catalase, both of which are linked to diabetes, but the extract of the plant has been shown to enhance the secretion of enzymes SOD and CAT ($p<0.001$). It has been proven to have a crucial role in the regulation of apoptosis because it lowers oxidative stress and similarly reduces the level of LPO ($p<0.01$). Additionally, the treated rat pancreas shows islets of Langerhans that are normal in number and size. No necrosis or reduction in size was seen. The current study conclusively shows that the phytoconstituents of *C. indica* have the potential to tackle long-term health complications and manage symptoms.

Introduction

Diabetes has a huge impact on the global population and researchers are striving to find a treatment that can cure this chronic metabolic disorder. It is estimated that the number of people affected by diabetes will rise 700 million by 2045. Most of the treatments are available only to manage the symptoms and suppress the progression of complications to a certain extent. Type 2 diabetes mellitus (DM) mostly affects the regulation of insulin, insulin deficit or resistance, which comes from changes in the metabolism of carbohydrates, proteins, and lipids brought on by a relative or absolute lack of insulin secretion and/or activity in the peripheral tissues.[1] In the next ten years, the predicted 5% of deaths worldwide attributable

***Corresponding Author:** Dr. Deepak K. Jha

Address: Department of Pharmacology, Karnataka College of Pharmacy, Bangalore, Karnataka, India.

Email ✉**:** deepakjha736@gmail.com

Tel.: +91-7411458812

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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to diabetes will rise by 50%.[2] The World Health Organization (WHO) estimates that 347 million people globally, primarily in developing nations, have diabetes. Cancer, heart disease, diabetes mellitus, and hypertension are chronic pathologies that have been treated and prevented with natural herbs and their derivatives.[3,4] More than 800 species have been researched up until 2014, and their hypoglycemic effects have been documented.[5] Ayurveda is managing the symptoms of diabetes and play a significant role as alternatives to conventional therapies because they are affordable and have fewer or no side effects. Among the various plants employed in the treatment of diabetes, *Coccinia indica* fruit is the one that has become popular globally to treat multiple diseases because of the different kind of phytoconstituents present over here. Plants contain resins, alkaloids, flavonoids, tyrosine, glutamic acid, histidine, threonine, phenylalanine, tannins, steroids, saponins, glycosides, phenols, and triterpenoids, etc.[6-9] It has been reported various medicinal values including skin disease, eruptions of smallpox, and scabies, healing ulcers, green fruits used as a vegetables and also used to remove eczema. The bark of this plant has been used for cathartic. Stem and leaves of this plant serve as a diabetes, and urinary tract infection (UTI) infection, are effective against chronic cough, and cold, and are good for asthma and bronchitis.

A potent Ayurvedic herb that offers numerous health benefits, reducing blood sugar, antioxidants, anti-inflammatory properties, and many more. Plants are effective herbal remedies and natural antioxidants in part due to their composition of anti-diabetic substances such as phytochemical(s), which enhance the function of pancreatic tissues by either reducing intestinal glucose absorption or increasing insulin secretion. We designed the study using *C. indica* fruits extracted with methanol in diabetic rats in order to achieve that goal. Hence, the aim of the study is to figure out the relationship between herbs and the complications of diabetes mellitus. Ayurveda can assist in controlling diabetes with the right food, lifestyle, herbs, and standard treatment.

Materials And Methods

Plant Material

The *C. indica* fruits were procured from Bangalore. Dr. Noorunnisa Begum was authenticated the plant specimen and it has been kept in FRLHT, Bangalore, India for future reference. And the reference number was FRLHT Acc. No 6395.

Preparation of Extract

The *C. indica* fruit was peeled, cut into small pieces, and dried for seven days at room temperature in the shade. The dried fruits were ground into a powder and strained (coarse 10/40). The powder was used for extraction.

Method of Extraction

Each 100 g of powder was extracted using 1000 mL of methanol in a reflux condenser over the course of three 7-hour cycles until the volume was decreased to half. To obtain a constant weight, the extract was filtered *via* Whatman paper and dried.[10-11]

Experimental Design

Research animals

Male Wistar rats weighing 150 to 200 g were considered for the current study and the study was performed as per the CPCSEA guidelines. The protocol was duly approved by the IAEC of the Karnataka College of Pharmacy, Bangalore, India and Sl. No (KCP/IAEC/11/22-23/01/22/12/22).

Dose selection

As reported previously (200 and 400 mg/kg, b.w.)[12]

Model for Type II Diabetes Mellitus

The rats were fed a high-sugar diet composition (normal pellets blended with 20% protein, 10% coconut oil, and 66% (w/v) fructose) for 10 weeks, and followed by 10% (w/v) of fructose, which was mixed in drinking water.[13] During the experiment, the body weight and blood glucose levels were noted. After completion of the study animals were anesthetized by phenobarbital sodium for blood collection by the method of cardiac puncture for the biochemical assay and later it was sacrificed and the pancreas was isolated, one part of the pancreas was homogenized for estimation of pancreatic insulin and antioxidants enzyme study, and another part of the pancreas was kept in 10% formalin for histological assessment. Samples were analyzed after centrifugation at 2500 rpm for 15 minutes.

Parameters

Body weight and fasting blood glucose level (Pre and Post-treatment). Serum and pancreatic insulin (Sandwich ELISA Assay)[11], the procedures were followed as per the manual instruction kits.

Antioxidant enzyme studies (CAT, LPO and SOD)[14,15]

Sacrificed the animals and isolated the part of the pancreas, removing residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH = 7.4). After weighing the tissue, mince it, and then homogenize it in PBS (9:1, volume: weight). And freeze-thaw the solution for further break up the cells. To obtain the supernatant, the homogenates are then centrifuged at 5000 g for 5 minutes and stored at -20°C.

Estimation of catalase

In 0.1 mL of homogenate + 1-mL of phosphate buffer, then added 0.4 mL of water and 0.5 mL of hydrogen peroxide (0.5%). After adding the above reagent incubate at 37°C for

Table 1: Groupings were done by the following manner, Where N = 8 animals in each group

01.	High sugar and fructose induced type 2 diabetes mellitus in rat's	Group I: Normal Control Group – Normal Saline 10 mL/kg, b.w., P.O.	6 rats
		Group II: Disease Control, Received Fed High sugar diet (Fructose 66% (w/v) + 10% coconut oil, and 20% protein) + Followed by, 10% (w/v) of fructose was given orally through water on a daily basis for 10 weeks.	8 rats
		Group III: Standard drug, Diabetic rats (Received High sugar diet for 10 weeks), After the confirmation of diabetic, treated by metformin 300 mg/kg/b.w, p.o., OD for 30 days.	8 rats
		Group IV: Test drug (Low dose), Diabetic rats (Received High sugar diet for 10 weeks), After the confirmation of diabetic, treated by *C. indica* extract 200 mg/kg,b.w p.o., OD for 30 days	8 rats
		Group V: Test drug (High dose), Diabetic rats (Received High sugar diet for 10 weeks), After the confirmation of diabetic, treated by *C. indica* extract 400 mg/kg,b.w p.o., OD for 30 days	8 rats

1-minute, then add 2 mL of potassium dichromate acetate solution, boil in a water bath for 15 minutes get a green color upon cooling, and get the absorbance at 570 nm.

Estimation of lipid peroxidation

Take 0.5 mL of homogenate + 2.5 mL TCA, 10% boil for 15 minutes, then centrifuge at 3000 rpm for 10 minutes. Added 2 mL of supernatant mixture + 1-mL 0.67% TBA again boiled for 15 minutes then got the absorbance at 532 nm.

Estimation of superoxide dismutase

To 2.78 mL NaCO3 buffer (0.05 mM, pH 10.2), 100 µL of 1 mM EDTA and 20 µL tissue supernatant was added and incubated at 30°C for 45 minutes. The reaction was initiated by adding 100 µL of adrenaline. The change in the absorbance was recorded at 480 nm for 3 minutes. Sucrose was used as a blank. The activity of SOD was expressed as U/mg of protein.

Determination of pro-inflammatory cytokines (TGF-beta, IL-6 and TNF-Alpha) from plasma[16]

Ref. test is based on solid phase sandwich ELISA, BD OptEIA™, Biosciences. Briefly, assay procedure: Using BSA as a reference protein, the total protein content was calculated using the *Lowry et al.* (1951) method. Samples must be stored at -20°C. Bring all reagents and samples to room temperature for 20 minutes before use. Wash buffer, standards, and biotin-labeled antibody working solutions were prepared within 30 minutes before the experiment as guided in the protocol kit. It is recommended to plot a standard curve for each test. Set standard, test samples, and control (blank) wells, each diluted at least half with sample dilution buffer, on the pre-coated plate, and then note their locations. Pipette out 50 µL of ELISA diluent into each well. In 50 µL of each sample and standard should be pipetted into the corresponding wells. Put plate sealer over the wells and let them sit at room temperature for two hours. Aspirate or decant the contents. Fill at least 300 µL of pre-mixed wash buffer into each cleaned well before decanting or aspirating. For a total of five washes, repeat the wash four times. Blot the plate with absorbent paper after the final wash to get rid of any leftover buffer. For effective performance, fluids must be completely removed. To each well, add 100 µL of the detection antibody. Put plate sealer over the wells and let them sit at room temperature for an hour. For the working reagent, pour the necessary amount of enzyme diluent into a clean tube or flask using a pipette. Enzyme concentrate in the desired amount (250x) should be added and thoroughly mixed. Add 48 µL of enzyme concentrate to 12 mL of enzyme diluent to fill a 96-well plate. Clean the wells then fill each one with 100 µL of the enzyme-working reagent. Put plate sealer over the wells and let them sit at room temperature for 30 minutes. Seven times in total, wash the wells. To each well, add 100 µL of TMB one-step substrate reagent. The plate should be left to sit at room temperature in the dark for 30 minutes. Within 30 minutes of halting the reaction, add 50 µL of stop solution to each well, and then measure the absorbance at 450 nm.

Histopathology Study:[17] Pancreas

The pancreas of each animal was isolated and has been cut into small pieces, and kept in 10% of formalin. Then the part was purified in running water for approximately 12 hours to remove the formalin and was accompanied by dehydrating with 70% rising in intensity isopropyl alcohol, 80% then 90% for 12 hours each and dehydrated with absolute alcohol. Further alcohol was extracted once using chloroform and paraffin infiltration to extract the chloroform. The clearance was carried out by using chloroform with twin modifications, each lasting 15 to 20 minutes. After paraffin infiltration, the parts of the tissue have been automatically processed. Melted Hard paraffin has been poured onto the L-shaped blocks. The tissue pieces were then rapidly plunged into the molten paraffin and cooled. The blocks have been sliced to parts concerning a thickness of 5 µm. The pieces were rendered by a micro slide, which used egg albumin i.e. sticking once. The parts were permitted to last for approximately one hour in an oven at 60°C. Paraffin melts and denatures the egg albumin, thereby fixing the slide tissue. Hematoxylin and eosin have been used to stain the tissue.

Statistical Analysis

The findings were expressed as Mean ± SEM (N = 8 rats/group). A one-way ANOVA and Tukey's multiple

Table 2: Percentage yield of the Fruits of *Coccinia indica* Extract

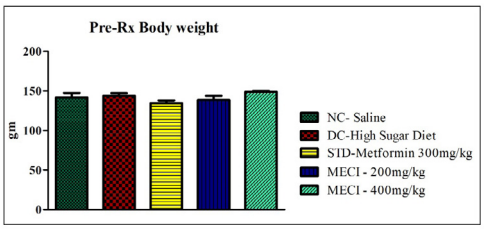
Plant extracted in	*Percentage yield*	*Color*	*Nature*
Methanol	12%	Black	Sticky

Body weight assessment of treated and untreated diabetic rats

comparison tests were used to analyze the data by using Graph Prism vs. 5 to compare the normal control group to all other groups, and a *p-value* is of less than 0.05 was considered significant.

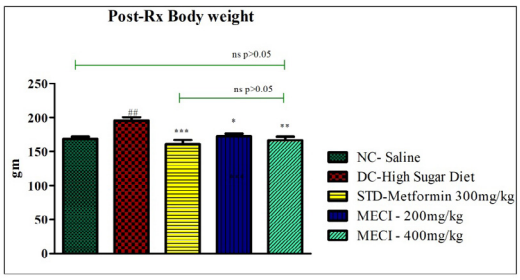
Results And Discussion

Traditional plants have been used worldwide for several years, and they may provide a natural starting point to find important opportunities for future drug development may be due to less expensive and less or no side effects. Over 800 traditional plants are available all over the world to claim that have anti-diabetic activity. Various ethnomedical surveys on medicinal plants have been conducted across the nation's divisions, districts, villages, and even hill tract and tribe regions. According to reports (*Dai and McNeill, 1995; Vrana and Kazdova, 1986; Suzuki et al., 1997; Katakam et al., 1998; Lee et al., 2002; Bunnag et al., 1997*), normal rats fed a fructose-enriched diet develop metabolic abnormalities like hyperglycemia, insulin resistance, hyper-insulinoma, and hypertriglyceridemia. *Thorburn et al. (1989), Tobey et al. (1982), and Zavaroni et al. (1982*), feeding a high-fructose diet can cause Type 2 diabetes linked to insulin resistance and hypertriglyceridemia. An excess of fructose disrupts the mechanisms for glucose absorption and metabolism. The fruits of *C. indica* were extracted by using methanol for the current study, and the yield was found to be 12%. (Tables 1 and 2). Fasting Blood glucose levels in diabetic rats were considerably lowered by the administration of a methanolic extract of *C. indica*. Loss of body weight is a hallmark of diabetes in rats; this loss of weight may be brought on by increased muscle waste and loss of tissue proteins and lipids. However, rats on high-sugar diets exhibited a considerable increase in body weight and a decrease in particularly MECI 400 mg/kg treated animals (Figs. 1 and 2). This may be attributable to MECI's protective effect in preventing muscle atrophy, which involves reversing gluconeogenesis, as well as to improvements in glucose uptake, insulin secretion, and glycemic control. The fact that Type II DM patients have resistant beta cells results in an insulin deficit. Due to enhanced insulin secretion or greater peripheral tissue utilization of glucose, the plant has demonstrated a significant drop in glucose levels when compared to their respective diabetic controls (Fig. 3). The plant had positive effects on serum insulin (Fig. 4) and pancreatic secretion (Fig. 5). The activation of the complement cascade, additional cytokines, and systemic inflammatory response are the end results of reactive oxygen species' peroxidation of membrane lipids, which releases hazardous byproducts.[18,19]



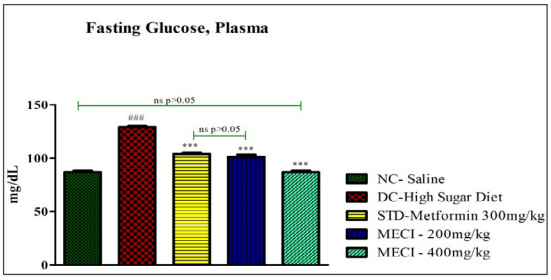
Values are expressed as Mean ± SEM, (n = 8 rats in each group). $^{ns}p > 0.05$ within the groups.

Fig. 1: Initial body weight



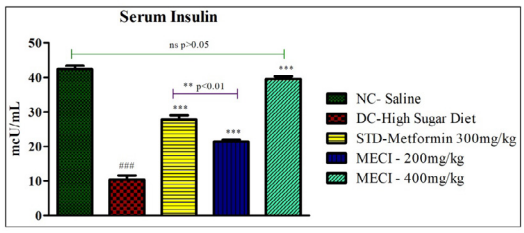
Values are expressed as Mean ± SEM, (n = 8 rats in each group). $^{\#\#}p < 0.01$ compared to Normal control, Saline, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, compared to disease control, high sugar diet. $^{ns}p > 0.05$ between the normal control vs MECI 400 mg/kg and metformin 300 mg/kg vs MECI 400 mg/kg

Fig. 2: Post treatment body weight



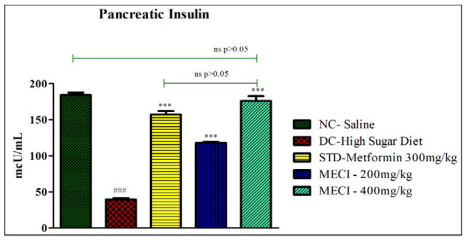
Values are expressed as mean ± SEM, (n = 8 rats in each group). $^{\#\#\#}p < 0.001$ compared to normal control, saline, $^{***}p < 0.001$ compared to disease control, high sugar diet. $^{ns}p > 0.05$ between the normal control vs MECI 400 mg/kg and metformin 300 mg/kg vs MECI 200 mg/kg

Fig. 3: Fasting blood glucose level



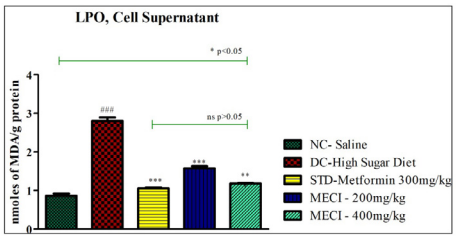
Values are expressed as Mean ± SEM, (n = 8 rats in each group). $^{\#\#\#}p < 0.001$ compared to normal control, saline, $^{***}p < 0.001$ compared to disease control, high sugar diet. $^{ns}p > 0.05$ between the normal control vs MECI 400 mg/kg and $^{**}p < 0.01$ metformin 300 mg/kg vs MECI 200 mg/kg

Fig. 4: Serum insulin



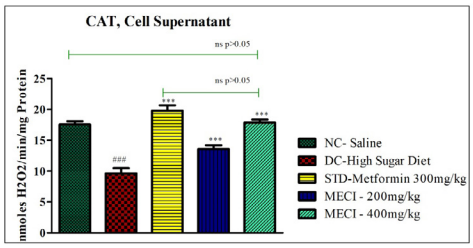
Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet. nsp > 0.05 between the normal control vs MECI 400 mg/kg and metformin 300 mg/kg vs MECI 400 mg/kg

Fig. 5: Pancreatic insulin



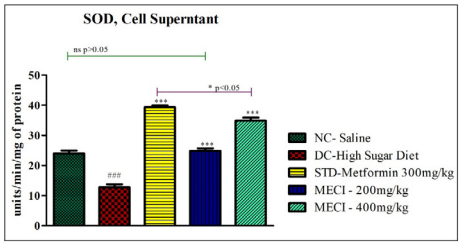
Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001, **p < 0.01 compared to disease control, high sugar diet. #p < 0.05 between the normal control vs MECI 400 mg/kg and nsp > 0.05 metformin 300 mg/kg vs MECI 400 mg/kg

Fig. 6: LPO, cell supernatant



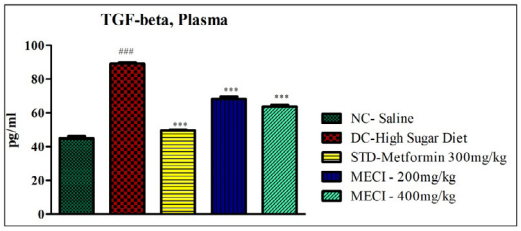
Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet. nsp > 0.05 between the normal control vs MECI 400 mg/kg and metformin 300 mg/kg vs MECI 400 mg/kg

Fig. 7: CAT, cell supernatant



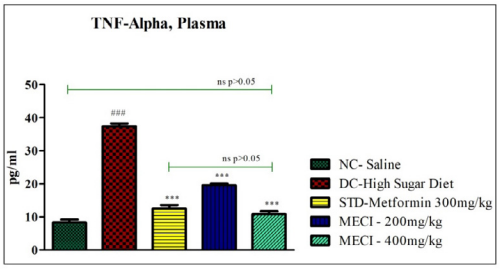
Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet. nsp > 0.05 between the normal control vs MECI 200 mg/kg and #p < 0.05 metformin 300 mg/kg vs MECI 400 mg/kg

Fig. 8: SOD, cell supernatant



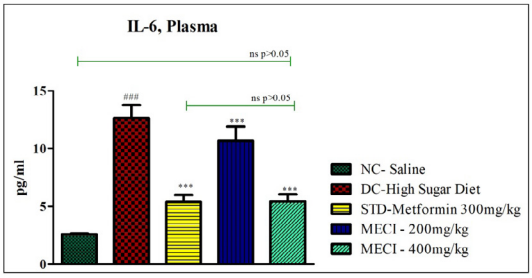
Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet

Fig. 9: TGF-beta, plasma



Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet. nsp > 0.05 between the normal control vs MECI 400mg/kg and metformin 300 mg/kg vs MECI 400 mg/kg

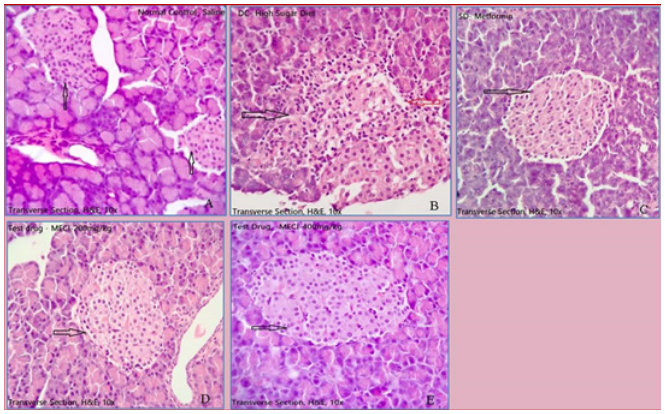
Fig. 10: TNF-alpha, plasma



Values are expressed as Mean ± SEM, (n = 8 rats in each group). ###p < 0.001 compared to normal control, saline, ***p < 0.001 compared to disease control, high sugar diet. nsp > 0.05 between the normal control vs MECI 400mg/kg and metformin 300mg/kg vs MECI 400mg/kg

Fig. 11: IL-6, plasma

In our investigation, diabetics had much higher LPO levels, but treatment appeared to stop the rise in LPO activity, as seen by significantly lower LPO concentrations (Fig. 6). According to the literature, SOD and CAT are the two components of the antioxidant defense.[20] The pancreas is indicative of tissue and systemic oxidative stress. Similarly, the levels of CAT (Fig. 7) and SOD (Fig. 8) were much lower in the diabetic rats, but they were significantly increased in the treated animals. Free radical production and development are greatly influenced by the immunological milieu, both at the systemic and micro-environmental levels. Cytokines are mediators that are essential for promoting the recruitment and activation of



A) Normal control – Normal Saline, Normal control rat pancreas shows islets of Langerhans that are normal in number and size (Black arrow). B) Diabetic- High sugar diet & fructose, diabetic control rat pancreas shows normal islets but few islets with lymphocytic infiltration (Red arrow) and beta cells depicted (Black arrow). C) Std. drug – Metformin, Std. drug rat pancreas shows islets of Langerhans that are normal in number and size. No necrosis or reduction in size seen (Black arrow). D) Test drug – MECI – 200 mg/kg, Test drug MECI – 200 mg/kg rat pancreas shows islets of Langerhans that are normal in number and size. No necrosis or reduction in size seen (Black arrow). E) Test drug – MECI – 400 mg/kg, Test drug MECI – 400 mg/kg rat pancreas shows islets of Langerhans that are normal in number and size. No necrosis or reduction in size seen (Black arrow).

Fig. 12: Histological assessments of normal and treated rat pancreas

particular subsets of leukocytes within the inflammatory milieu. They also serve as prognostic indicators for inflammation. The immune profile is dynamic in nature and can change with disease status and treatments. To better understand how the immune milieu changes during illness and therapy, cytokines like IL-6, TNF-alpha, and TGF-beta have been considered. TGF-beta is known to be associated with immunosuppressive activity and is correlated with a reduced immune response. TNF-alpha and IL6 are indicators of systemic inflammation. Increases in serum levels of these cytokines are associated with an inflammatory milieu and poorer overall outcomes. Chronic infections and inflammatory disorders are more common in people with hyperglycemia, and high pre-treatment TGF-β levels were linked to poor prognoses. With the administration of *C. indica*, it has been shown that lower levels of these pro-cytokines (Figs 9-11) are an indication of immunoprotective benefits and improved outcomes. Overall, systemic immune suppression and inflammation appeared to have improved significantly. An improvement in markers points to a decrease in inflammation. The methanolic extract of *C. indica* observed normal islets, no necrosis, and a reduction in islet cell degeneration. Histological images of rats fed a high-sugar diet exhibited degeneration of islets and disruption of normal islets with lymphocytic infiltration (Fig. 12). Cell degeneration can be reversed by giving an effective glycemic control drug to allow it to resume normal function. Regular monitoring of these markers can help determine how the body responds to the disease and treatments in terms of the immune status, which can help in tailoring the primary and adjuvant treatment options.

Conclusion

The results of the study showed that the methanolic extract of *C. indica* fruit had potent anti-diabetic efficacy. Reduced body mass, fasting blood glucose levels, improved serum, and pancreatic hormone insulin may all help to minimize long-term complications. The anti-inflammatory cytokines; TGF-beta, TNF-alpha, and IL-6, were decreased after treatment with *C. indica*. The extract decreases oxidative stress by increasing the release of enzymes SOD and CAT and decreasing LPO levels. Diabetic-treated rats exhibit normal in size and number of islets of Langerhans. There is no evidence of necrosis or shrinkage of the beta cell mass. These outcomes all serve as strong evidence and precedent for *C. indica* as an anti-hyperglycemic candidate.

Acknowledgement

The authors express their thanks to Karnataka College of Pharmacy, Bangalore, for extending the infrastructure for carrying out the research.

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HOW TO CITE THIS ARTICLE: Ali AAM, Rajashekhar U, Jha DK. An Investigation of Type II Anti-diabetic Activity of Biologically Active Phytochemical(s) of *Coccinia indica* Fruits in High Sugar Diet-Induced Diabetic Rats. Int. J. Pharm. Sci. Drug Res. 2024;16(1):52-58. **DOI:** 10.25004/IJPSDR.2024.160108