RESEARCH ARTICLE

Effects of Methanol Extract of *Corchorus olitorius* Cultivated in Iraq on High Fat Diet plus Streptozotocin-induced Type II Diabetes in Rats

Noor H. Swayeh[1]*, Haitham M. Kadhim[2]

[1]*Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq*
[2]*Department of Pharmacology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq*

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ABSTRACT

Background: Diabetes mellitus (DM) is a long-lasting clinical syndrome characterized by raised blood glucose leading to numerous other complications.

Objective: To study the phytochemical, anti-hyperglycemic, anti-hyperlipidimic and histopathological effects of methanol extract of *Corchorus olitorius* cultivated in Iraq against induced type II diabetes in rats.

Methods: *C. olitorius* leaves extracted with 60% methanol then phytochemical analysis done, forty male rats separated into four groups ten in each the first not received any treatment as a normal control, remaining rats were induced diabetes type 2 by 28 days feeding of high fat diet followed by single intraperitoneal injection of streptozotocin 35 mg/kg, ten of them not received treatment served as diabetics, third and fourth groups received oral metformin (MET) 100 mg/kg and *C. olitorius* methanol extract (COME) 400 mg/kg respectively for 28 days. Blood samples collected at day 29 for biochemical study of lipid profile and glycemic indices as well as assessment of histological effects on pancreatic tissue sections after scarification.

Results: *C. olitorius* methanol extract contains anthraquinons, flavonoids, saponines and steroids. Treatment with MET or COME significantly reduced the elevated levels of fasting blood glucose, glycated hemoglobin A1, homeostasis model assessment of insulin resistance, total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein, while, enhance the reduced levels of adiponectin and high density lipoprotein, also COME enhanced insulin release. Histopathological picture of pancreatic tissues of COME or MET treated rats improved than diabetics.

Conclusion: Methanol extract of *C. olitorius* cultivated in Iraq has anti-hyperglycemic, insulin releasing and sensitizing, anti-hyperlipidimic and pancreatic tissue protective effects in type 2 diabetic (T2D) rats.

Keywords: *Corchorus olitorius*, Hyperglycemia, Hyperlipidemia, Methanol extract, Phytochemical, Streptozotocin, Type 2 diabetes.

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INTRODUCTION

Diabetes mellitus is a long-lasting clinical syndrome characterized by high blood glucose because of reduced or absent insulin secretion, which is often accompanied by reduced sensitivity to its work.[1] Prevalence of diabetes in Iraq was 6.9%, according to the International Diabetes Federation 2017.[2] Type 2 diabetes affects 90–95% of diabetics, so the global prevalence of diabetes and impaired glucose tolerance in adults has significantly increased in recent decades, and persistently high blood glucose levels cause generalized vascular damage that can affect many tissues, most notably the heart, eyes, kidneys, and nerves, leading to a variety of complications.[3,4] Hyperglycemia causes free radical production, and oxidative stress is a major contributor to cellular damage.[5,6] Diabetes mellitus management is regarded as a global problem because, as the disease progresses, monotherapy fails to provide the predicted response, necessitating the use of multiple drug combinations with increased risk of side effects, with this knowledge, there is an urgent need to shift to nontoxic, high efficacy, low-cost options such as herbs, which are considered excellent candidates for oral therapy.[7,8] According to research, many human diseases can be protected against oxidative stress by utilizing the antioxidant capacities of plants; as a result, flavonoids and other polyphenolic compounds have

**Author for Correspondence: noorhassoon@gmail.com*

piqued the most interest.[9,10] *Corchorus olitorius*, also known as Nalta jute, tossa jute, Jew's mallow and bush okra,[11] is a cultivated plant in Iraq -known as "molokhiya''- and Jute leaves are utilized as a food source, its grown in other Middle Eastern and African countries such as Egypt, Tunisia, Nigeria, Southern Asia, Japan, India, China, Lebanon, Palestine, Syria and Jordan. Antioxidants like as ascorbic acid, vitamin E, beta carotene, alpha tocopherol, glutathione, and phenols have been discovered in the leaves of various Jute species.[12] Its leaves are also rich in fatty acids, other vitamins, minerals, and mucilaginous polysaccharides, which have many medical actions such as anti-oxidant, anti-inflammatory,[13] anticancer, antidiabetic, anti-obesity, gastroprotective, antinociceptive, anticonvulsive, antihypertensive, antimicrobial and antiviral activity.[14-16] As a result of this background, *Corchorus olitorius*, which is grown in Iraq, was chosen for the current study.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) and metformin (Met) from sigma Aldrich/ Germany. Serum adiponectin (ADP) and insulin (INS) elisa kits were purchased from bioassay technology laboratory/ China, any other chemicals used of analytical grade purity.

Analysis of Boichemicals

Fast blood glucose (FBG) determined by glucometer Accu-Check Roche Diagnostics/ Germany, biochemical tests of glycated hemoglobin A1 (HbA1c), total cholesterol (T-Chol), triglycerides (TGs), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) done utilizing an auto-analyzer seimens/ Germany, homeostasis model assessment of insulin resistance (HOMA-IR) determined according to the following formula (Matthews *et al*., 1985): HOMA-IR = Fasting glucose(mg/dl) × Fasting insulin (uU/mL)/405

Plant Collection and Extraction Process

Fresh *C. olitorius* plants were collected from a local market in Baghdad City, the plant was recognized and authenticated at the Department of Biology, College of Sciences, University of Baghdad, the leaves were rinsed properly, dried in the shade, and ground to a fine powder using an electrical grinder, and then (1150 g) were macerated thrice (24 hours each time) in 60% methanol (1:5 w/v) with continuous shaking from time to time, the crude extract was then filtered using solvent-wetted Whatman no. 1 filter paper, and the filtrate was dried[17] to semisolid material by overnight incubation at 40 C in a vacuum with a final weight of (209.7 g) and a yield of 18.97% estimated, kept refrigerated at 4 °C in parafilm-sealed, dark containers until needed later in the study. The appropriate doses were reconstituted in distilled water (DW) just before oral delivery.

Preliminary Phytochemical Study of Crude Extract

Harborne's standard techniques were followed to identify the presence of alkaloids, flavonoids, steroids, anthraquinones, cardiac glycosides and saponins in the *C. olitorius* methanol crude extract (COME), then the preliminary study done.

Diabetes Induction

The approach described by Srinivasan *et al*., 2005[18] was used to create diabetes in 30 rats with minor modifications: For 4 weeks, rats were fed a high-fat diet (HFD) obtained from Research diet with a total kcal value of 4.73 kcal/g (45 kcal %energy as fat, 20 kcal % protein, and 35 kcal % carbohydrate). The rats were then fasted overnight and given a single intraperitoneal injection of streptozocin 35 mg/kg prepared in 0.1M ice-cold Citrate buffer at pH 4.57.[19] Animals were given a 5% glucose solution to drink overnight to offset drug-induced hypoglycemia.[20] The control group was given the same amount of solvent (citrate buffer) as the Ip injection as well as a standard pellet meal. The diabetes status of the animals was assessed 5 days after STZ induction; those with FBG levels of ≥200 mg/dL were considered diabetic, and they were retained on the HFD and used in the study.[21]

Animals and Study Design

The current experiment was conducted as an animal research study in the animal house of the biotechnology research center, Al-Nahrain University from June 2020 to September 2021. All study experiments were carried out in accordance with the protocol approved by the Institutional Review Board of the College of Medicine, Al-Nahrain University. At the age of 10 to 12 weeks, forty presumably healthy Albino Wister male rats weighing 250 to 350 g were kept in typical plastic cages with a 12-hour/12-hour light/dark cycle, an ambient temperature of 22 to 25°C, fresh water, and a regular chow diet (ad libitum). The rats were given a week to adjust to their new environment before the experiment began. The animals were randomly divided into four groups (GPs) of ten rats each, with GP1 serving as a normal control and receiving equivalent doses of used solvents, while the other three GPs induced diabetes 2, GP2 serving as a diabetic control given (DW) orally, and GP3 receiving 100mg/kg metformin orally gavaged and GP4 received 400 mg/kg Iraqi *C. olitorius* methanol extract,[22] the therapy session lasted 28 days.

Sacrifice and Collection of Samples

On day 29 of the study, the rats were sacrificed by euthanizing them with diethyl ether anesthesia after an overnight fast, and sample was drawn directly from the heart for chemical analysis, while the pancreas was washed with 0.9% NaCl and kept in 10% formalin for dissection and histopathological examination.

Statistical Analysis

The mean and standard deviation were used to express quantitative variables, and one-way ANOVA was used to investigate the difference in mean of numeric variables between more than two groups, followed by a post hoc least significant difference test to determine the mean difference between any two groups. p 0.05 was chosen as the criterion of significance, using Statistical package for social sciences SPSS version 23.[23]

RESULTS

Table 1 shows the preliminary phytochemical analysis results, which revealed the presence of anthraquinons, flavonoids, saponines, and steroids while alkaloids and cardioactive glycosides were lacking from (COME). Table 2 revealed that, when comparing the diabetes group to the normal control animals, there was a highly significant decline $p \leq 0.001$ in body weight gain, S.INS, and S.ADP levels, but a highly significant elevation $p \leq 0.001$ in FBG, HbA1c, and HOMA-IR. Comparing diabetic group to Met and COME groups its showed a highly significant elevation $p \leq 0.001$ in FBG, HbA1c, also high significant increase $p \leq 0.001$ in HOMA-IR compared to MET group with significant $p \leq 0.05$ increase compared to COME, but a significantly high lowering $p \leq 0.001$ in S.ADP compared to COME and significantly low level $p \leq 0.05$ compared to Met, Serum insulin showed a significant increase in COME compared to diabetic rats, as well as no significant differ in relation to both concerning the body weight gain. Comparing Met to COME group the first showed a high significantly lower $p \leq 0.001$ FBG and HOMA-IR levels with significant $p \leq 0.05$ decrease in S.ADP. Table 3 revealed that there were a highly significant increase $p \leq 0.001$ in all serum lipid markers (T-Chol, TGs, LDL, VLDL) when diabetic group compared to all groups, except for S.HDL which showed a highly significant decrease $p \leq 0.001$ when diabetic group compared to normal rats, with significantly lower levels $p \leq 0.05$ than Met and COME groups. The examination of histopathological changes revealed that compared to normal group, diabetic

Table 1: Phytochemical compounds in Iraqi *C. olitorius* methanol crude extract

Compound	*C. olitorius*
Alkaloids	-
Anthraquinons	+
Cardio-active glycosides	-
Flavonoids	+
Saponines	+
Steroids	+

+ denote the presence
- denote the absence

Table 2: Comparison on body weight and glycemic parameters between groups

Type	*Mean ± SD normal*	*Mean ± SD diabetic*	*Mean ± SD metformin*	*Mean ± SD jute*
WT 1 (g)	288.2 ± 40.74	228.2 ± 21.45	233.4 ± 25.39	291.6 ± 23.63
WT 2 (g)	329.2 ± 38.81	247.3 ± 25.12	251.7 ± 26.08	307.8 ± 22.77
Body weight gain (g)	41 ± 12.71 a*	19.1 ± 5.52 b	18.3 ± 2.62 B	16.2 ± 3.79 b
FBG (mg/dL)	101.30 ± 13.87 a*	408.90 ± 72.33 b	140.50 ± 13.53 c*	271.50 ± 105.16 d*
H bA1c %	3.25 ± 0.26 a*	4.98 ± 0.46 b	3.62 ± 0.46 c*	4.23 ± 0.55 d*
S. insulin (mU/L)	6.60 ± 0.87 a*	4.93 ± 1.62 b	5.13 ± 0.36 Bc	5.72 ± 0.28 c
HOMA-IR	1.47 ± 0.23 a*	4.37 ± 1.12 b	1.59 ± 0.15 c*	3.42 ± 1.24 d
S. adiponectin (mg/dl)	33.96 ± 3.66 a*	11.84 ± 4.10 b	18.50 ± 9.20 C	24.24 ± 4.17 d*

Small letters for groups comparison; similar letters indicate no difference; different letters indicate significant differences; SD: Standard deviation. Significance level at $p \leq 0.05$. * denote highly significant differ $p \leq 0.001$ compared to diabetic group. Red color denotes highly significant differ $p \leq 0.001$ compared to metformin group.

Table 3: Comparison between groups in lipid markers

Type	*Mean ± SD normal*	*Mean ± SD diabetic*	*Mean ± SD metformin*	*Mean ± SD jute*
S.T-Chol (mg/dL)	65.97 ± 6.40 a*	123.01 ± 23.77 b	69.49 ± 4.24 c*	73.05 ± 9.14 c*
S.TGs (mg/dL)	78.8 ± 2.85 a*	112.3 ± 15.89 b	83.0 ± 9.20 c*	85.4 ± 8.28 c*
S.LDL (mg/dL)	19.29 ± 8.71 a*	85.49 ± 23.88 b	32.11 ± 4.93 c*	36.07 ± 8.77 c*
S.VLDL (mg/dL)	15.76 ± 0.57 a*	22.46 ± 3.17 b	16.60 ± 1.84 c*	17.08 ± 1.65 c*
S.HDL (mg/dL)	30.92 ± 4.34 a*	15.06 ± 4.11 b	20.78 ± 4.87 c	19.9 ± 0.81 c

Small letters for comparison between groups; similar letters for no difference; different letters for significant differences; SD: Standard deviation. Significance level at $p \leq 0.05$. * denote highly significant differ $p \leq 0.001$ compared to diabetic group.

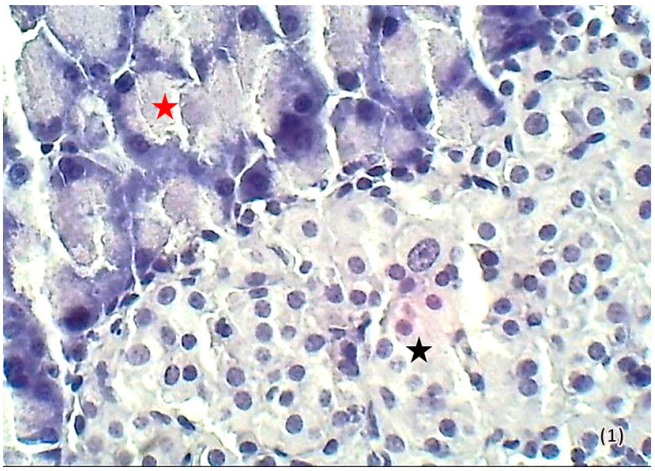

Figure 1: Section of pancreas (normal Gp) shows: pancreatic acinus (Red asterisk) & pancreatic islet (Black asterisk), H&E stain. 400x.

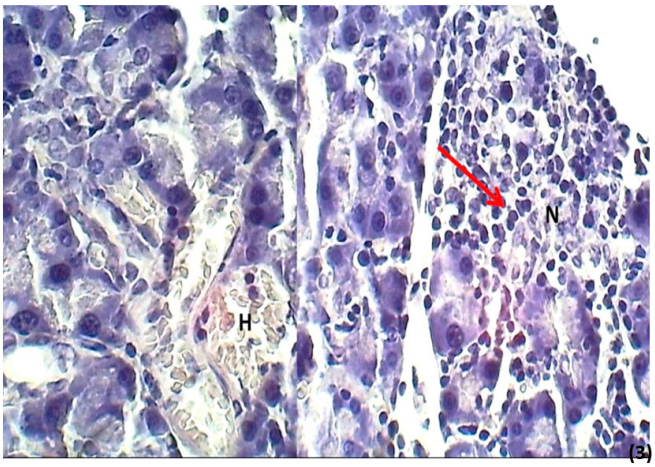

Figure 2: Section of pancreas (Diabetic Gp) shows: deterioration of acini with hemorrhagic focus (H) focal infiltration of mononuclear lymphocytes (Red arrow) and necrotic cells (N), H&E stain. 400x.

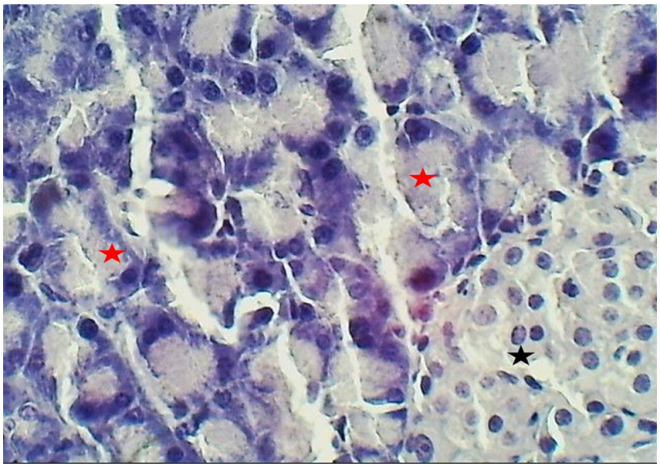

Figure 3: Section of pancreas (MET Gp) shows: normal acinar cells (Red asterisks) normal islet cells (Black asterisks), H&E stain. 400x.

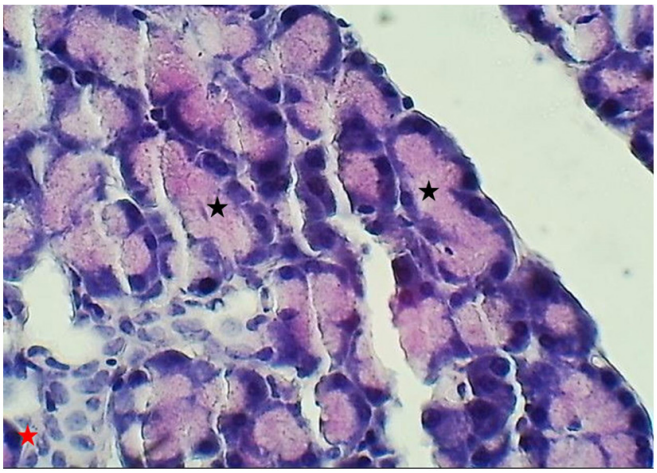

Figure 4: Section of pancreas (COME Gp) shows: normal acinar cells (Black asterisks) normal islet cells (Red asterisks), H&E stain. 400x.

group pancreatic sections characterized by focal necrosis of pancreatic islet surrounded by infiltrated lymphocytes, there were multiple focal hemorrhage and deterioration of pancreatic acini, while histological sections of pancreas from other two groups showed improved appearance to seems like normal (Figures 1–4).

DISCUSSION

When paired with low dosage STZ, a high-fat diet is one of the unhealthy lifestyles that can promote hyperlipidemia, hyperglycemia, insulin resistance, and mitochondrial dysfunction to resemble the symptoms of type II diabetes in humans. The current study found that after STZ administration, animals had higher FBG and HbA1c levels but lower INS levels, which is due to the selective toxicity on pancreatic β-cells caused by DNA alkylation, which results in a decrease in insulin levels and an alteration of glucose metabolism and utilization, resulting in hyperglycemia; additionally, STZ intracellular metabolism produces nitric oxide free radicals, which exacerbate these effects. (Sabitha *et al*., 2011).

Metformin and COME treatment effectively lowered elevated FBG and HbA1c levels, with increased insulin levels in the case of COME. Metformin has previously been shown to lower HbA1c and FBG in T2D. Metformin's activation of adenosine monophosphate kinase suppresses liver glucose production while increasing skeletal muscle glucose uptake, reduces insulin resistance by increasing insulin receptor tyrosine kinase activity, glycogen synthesis, and recruitment and activity of glucose transporters (GLUT4), and there was evidence of reduced intestinal glucose absorption.[24-26] As demonstrated in this experiment, *Corchorus olitorius* extract contains a variety of phytochemicals with known hypoglycemic properties such as anthraquinones, saponines, flavonoids and steroids and the extract's anti-diabetic ability may be related to them. Previous researchs found that diabetic rats fed a methanolic extract of *Corchorus olitorius* had lower blood glucose and HbA1c levels.[27,28] The presence of phenolic chemicals in an extract of C. olitorius leaves has been shown to strongly inhibit α-glucosidase but only moderately inhibit α-amylase, this may contribute to its glucose-lowering actions by delaying starch digestion and physical adsorption

of glucose, limiting its diffusion into the intestinal lumen and enhancing glucose absorption in peripheral tissues in addition to increasing insulin levels by promoting insulin release from the remaining β-cells of the pancreas that were not damaged by STZ and restoring the normal architecture of pancreatic lobule in addition to its anti-inflammatory and anti-oxidant actions. Treatment with either MET or COME corrects the abnormal levels of serum adiponectin and HOMA-IR, indicating reduced insulin resistance and increased insulin sensitivity. By inhibiting hepatic gluconeogenesis and promoting skeletal muscle glucose transport, pancreatic insulin synthesis, and hepatic and skeletal muscle fatty acid oxidation as well as the regulation of inflammatory signals adiponectin has been shown to lower the chance of developing type 2 diabetes. Metformin has previously been associated to statistically significant increases in adiponectin.[10-21] In line with the findings of this investigation, serum adiponectin levels in *C. olitorius* extract-treated rats were greater than in HFD control rats as previously reported. Hyperlipidemia is frequent in diabetic adults and shows a deficiency in insulin action caused by inadequate secretion, resistance, or both. The current study discovered a highly significant increase in T-Chol, TGs, LDL, and VLDL levels, while HDL showed a highly significant decrease in its mean in diabetic rats compared to normal animals, and treatment with MET or COME improved the lipid profile by elevating HDL while lowering harmful lipid levels (T-Chol, TGs, LDL, VLDL), comparable to the current study, previously found that streptozotocin cause hyperlipidemia in diabetic rats. In a previous study conducted by Airaodion *et al*. at 2019, *Corchorus olitorius* leaves methanolic extract was found to dramatically lower FBG, total cholesterol, LDL, and TGs while increasing HDL in diabetic hyperlipidemic rats. Suppression of cholesterol and triglyceride synthesis,[24-28] inhibition of pancreatic lipase activity[29] as well as boosting the expression of PPAR[30] have been proposed as lipid-lowering mechanisms. Previously, it was discovered that metformin lowers LDL and TG levels while boosting HDL levels in statin-naive persons with newly diagnosed T2DM, this is due to metformin decreasing the hepatic conversion of free fatty acids to lipoprotein precursors by improving insulin sensitivity (Lin *et al*., 2018). These findings directly support *Corchorus olitorius'* ability to lower hyperlipidemia in diabetes and thus protect against diabetes-related cardiovascular complications, and these effects were indeed related to its phytochemicals, which have many mechanisms for lipid lowering in addition to anti-oxidant and anti-inflammatory effects. In the current study, HFD+STZ induced diabetic rats had decreased pancreatic islet size, damaged β-cell population, and necrotic degenerative changes, whereas diabetic rats treated with the study's agents had shifts the pancreatic tissue picture to reclaim to normal, which is consistent with their effect on enhancing glycemic control as well as enhancing lipid profile. Many studies have demonstrated that STZ-induced hyperglycemic rats have fewer β-cells, but phytochemical-treated rats had more pancreatic β-cell populations, also a previous study reported that the extracts of *C. olitorius* leaves have been found to produce considerable improvement and enhance the number, size, and density of viable β-cells by restricting the apoptotic signaling pathways via the presence of a significant amount of phenolic chemicals and flavonoids in the extract. In addition, a previous study found that metformin-treated diabetic rats had weaker protective effects histologically in the pancreas, kidney, liver, brain, heart, spleen, and testicular tissues when compared to *Cistus laurifolius* extract, which had a higher increase in overall antioxidant potential than metformin.

CONCLUSION

In conclusion, a 400mg/kg dose of methanol extract of *C. olitorius* cultivated in Iraq has anti-hyperglycemic, enhanced insulin secretion and sensitization, anti-hyperlipidemic, and improved histopathological picture of pancreas because of phytochemicals it contains such as flavonoids, saponins, anthraquinones, and steroids.

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