

Original Research Article

Ameliorating Attribution of Diet Mixed *CocciniaIndica* Leaf Extracts in Experimentally Alloxan-Induced Diabetic WistarRats in a Dose Dependent Approach.

Abstract

Context: Some plants have been claimed to have anti-diabetic potential. *Cocciniaindica* is one of them. The plant has been used since ancient times for treating diabetes mellitus in the herbal medicine system.

Objective:The purpose of the present study was to identify the therapeutic effect of leaf extract of *Cocciniaindica* as well as to determine its safety profile so that the plant material could be used to ameliorate the diabetic condition.

Materials and methods: The extract of *Cocciniaindica* were soaked with ethanol. Alloxan monohydrate induced diabetic rats were fed with ethanol extract of *C.indica* individually.

Results:Diabetes was induced in rats by intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/Kg bodyweight and ethanolic extract of leaves of *Cocciniaindica* was fed to the rats at dose of 500, 750, 1000 mg/kg. We measured blood glucose level on diabetic and non-diabetic rats throughout the study time. After measuring blood glucose level, it was detected that the all doses of *Cocciniaindica* diminish the abnormally elevated blood glucose level of rats. However, only does 750 mg/kg significantly ($p < 0.05$) reduce the blood sugar. So, medium dose provided the highest efficacy due to higher consumption of food and dosage intake. Whereas in low dosage, rats consumed the food but the dosage was lesser. On the contrary, in high dosage, due to bitter taste of the extract rats consumed less food and dosage.

Conclusion:The study concludes that both metformin and leaf extract of *Cocciniaindica* improved the pathological condition induced by diabetes. It substantially demonstrates that the extract of leaves of *Cocciniaindica* could be used as a considerable therapy to treat diabetes.

Keywords: Diabetes mellitus, *Cocciniaindica*, blood sugar, alloxan, herbal medicine

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder depicted by uplifted levels of plasma glucose. Diabetes has arrived epidemic level, affecting over 400 million people worldwide [1], and it is also becoming pertinent in developing countries. It is referred to as a silent killer due to its annual contribution to precisely 18% of all deaths in the United States among patients of 25 and older.

The estimated universal healthcare costs cognate with the disease was 673 billion US dollars [2]. Correspondingly, it is estimated that by 2040, the populace living with diabetes will aggrandize by approximately 55% [3]. Heart disease is the paramount cause of fatality in patients with diabetes and has a mortality rate six times superior among male patients with diabetes and four times greater among female patients with diabetes. Other intricacies of diabetes include retinopathy, neuropathy and nephropathy [4]. In the bygone decade, an augmented risk of some cancers, such as pancreatic, liver, colorectal, endometrial and breast cancer has been appended to the conventional vascular intricacies of diabetes [5].

Cocciniaindica (Cucurbitaceae), widely known as little gourd and known as 'Kovai,' to indigenous Indian subcontinent. It grows abundantly and wildly throughout

Indiansubcontinent. Indigenous peopleutilizenumerous parts of the plant to get medicament from diabetesmellitus.Previous scientific studiesexhibited thatextract of Cocciniaindicaflauntshepatoprotective[6],anti-inflammatoryand anti-nociceptive [7] hypolipidemic[8]. It is widely used in traditional treatment of diabetes [9].Scientific investigations have corroborated the efficacy of leaf extracts in progress of diabetic conditions [10]. In the present study,we have investigated the medicinal effects of Cocciniaindicaagainst Alloxan-induced diabetes in animal models.

Material and methods

Animals

Wistar rats(200-250 gm) were housed in ambient roomtemperature ($24 \pm 2^\circ \text{C}$) and approximate humidity ($50 \pm 5\%$),maintained at 12:12 h dark–light cycle. Food and water wereavailable ad libitum. Rats were kept individually into different cages. All protocols were authorized by InstitutionalAnimal Ethics Committee of University of Dhaka and carried out under strict acquiescencewith Committee for the Purpose of Control and Supervision ofExperiments on Animals.

Reagents

Alloxan hydrate [$\text{OC}(\text{N}(\text{H})\text{CO})_2\text{C}(\text{OH})_2$]and Ethanol were purchased from Sigma Aldrich. SCPT, SGOT, HDL, LDL Total Cholesterol, Triglyceride , Urea and Creatinine kits was purchased from Plasmatec Laboratory, UK. Glucometer strips were taken from Roche Diagnostics. All other reagents used belonged to analytical grade.

Preparation of diet

Diet was freshly prepared. Cocciniaindica leaves powder were blended into the diet pulping by substituting same quantities of starch at 10% and 5% levels, discretely.As rates were kept into different cages, they had to be fed individually. On average, a rat eats 20 gm food. In every 10gm food pulp, respective amount of extract mg was carefully blended with it. Next, sufficient quantity dietary supplement was added to make mass of 20 gm.

Comment [A1]: Add plant name in italics

Plant material and extraction

Plant leaves (1 kg) of Cocciniaindica were washed carefully with distilledwater to remove dirt and soil, taken in drier.After drying, the plant materials were milled topowder, and then it was defatted with petroleum ether and extracted with 70% of ethanol by cold mediumfor 48 hours. The extract was dissociated by the filtration andconcentrated on vacuum evaporator.Also, a dark semi-solidmaterial was obtained. The dried plant materials stored in airtight containers in 3°C in a refrigerator until use.

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Comment [A3]: Mention % weight of extract

Statistical Analysis

Entire results were analyzed by One-way anova test using SPSS Software. Here P value less than 0.05 ($p < 0.05$) is considered as statistically significant.

Result and Discussion:

Influence on Body Weight: Initial and final body weight level of 14 groups is displayed in the below mentioned graph.

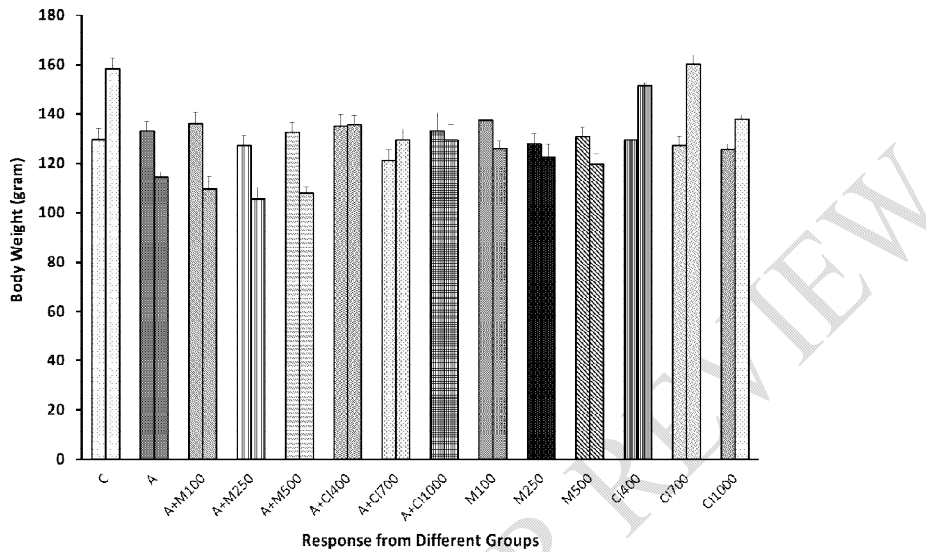


Figure 1: Body weight of rats before the initiation and after the termination of the experiment. Values were expressed as mean \pm SD (C = control group, A = alloxan-induced group, M = metformin, A + M = alloxan + metformin, A + CI = alloxan + Coccinia, and CI = C. indica).

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As observed from Figure 2, after end of the study, due to cellular necrosis of β pancreatic cells, alloxan in positive control rats exhibited a significant decrease in weight. In group (A + M100), (A + M250), and (A + M500) the combined use of metformin and alloxan were not able to reverse the effects of alloxan and the body weight of the rats at the end of the study as a result the body weight significantly decreased. Oppositely, the rats in group (A + CI400), (A + CI700), and (A + CI1000) that were treated with alloxan and plant extract in combination showed an increase in body weight due to the inhibitory effect on alloxan by the plant extract but the effect on group (A + CI700) was significant. As rodents of group (A + CI700) had ingested food with enough extract in comparison with low dose and high dose group (A + CI400) and (A + CI1000) respectively the effect observed was significant. Single extract treated rats in group CI400, CI700 and CI1000 had also significantly increased the body weight of the rats.

Anti-hyperglycemic Test: Blood glucose level of 14 groups of rodents is illustrated in the following graph.

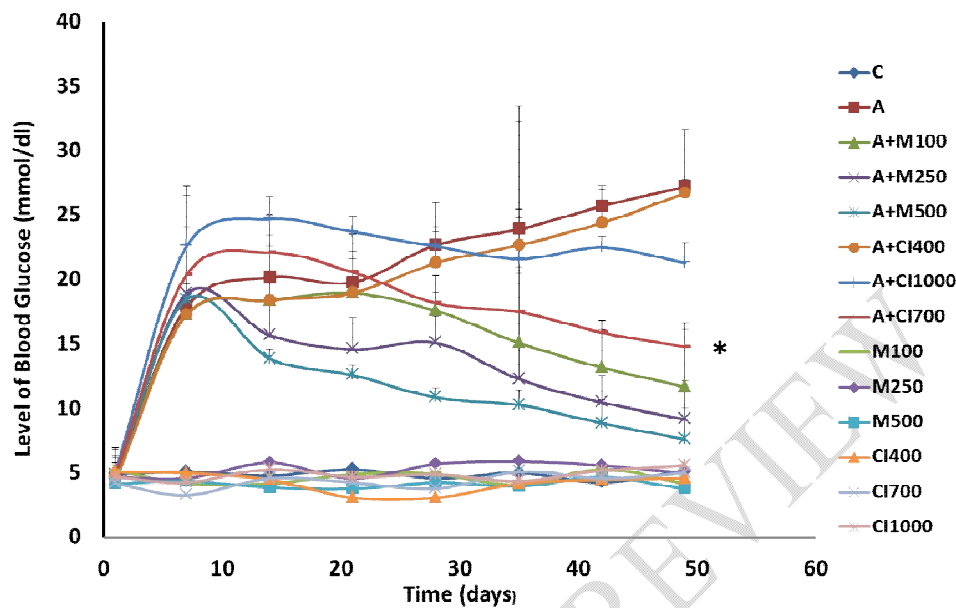


Figure 2: Blood glucose level of rats belonging to fourteen groups throughout receiving respective treatments. Values were expressed as mean \pm SD (n = 10/group). *p < 0.05 indicate significant differences from the disease group (C = control group, A = alloxan-induced group, M = metformin, and CI = C. indica).

As expressed in figure 2, Rats were divided into fourteen groups and were treated with different combination of drugs and extracts. Single drug or extract treatments with drugs or extracts kept the blood glucose level similar with the control group without lower it than normal from group M100 to CI1000. The positive control rats went under cellular necrosis of β -pancreatic cells due to administration of alloxan showed a significant increase blood glucose.

Alloxan having a higher availability was used on rodents to induce type-1 diabetes. Extract of the plant in alloxan induced diabetic rats of group A + CI700 reduced the blood glucose level significantly. However, *Coccinia* extract did not show low and high dose in reducing blood glucose level of alloxan reduced diabetic rats. The curve also represented that the *Coccinia* extract did not function in a dose dependent manner. This could be reasoned by as after end of treatment, it was observed low dose (500mg/kg) rat eats 18.70 gm of food in average and medium dose rat (750mg/kg) eats 18.10 gm of food. Whereas high dose rat (1000mg/kg) eats 11.6 gm of food. It indicates low dose and medium dose ate up most of the food. But high dose could not eat half of the food properly. Because in high dose, due to extreme bitter taste rat could not eat sufficiently. On the other hand, medium and low dose had lesser bitter taste. However, it can be observed in group A + CI700 that a higher dose of plants extract is required to lower glucose level in alloxan induced diabetic rats but in group A + M100, A + M250 and A + M500 amount of metformin was much lower to show an effective therapeutic effect decreasing blood glucose level in alloxan induced diabetic rats.

A similar study was conducted in streptozotocin induced diabetic Wister strain albino rats with methanol extract was given for 10 days. According to the study, it had similar effects

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showing decrease in glucose level. However, it exhibited an increase in dose further lowered the glucose level.^[11]

Kidney Function Test: Creatinine and urea level of 14 groups of rodents after treatment is demonstrated in the graph below.

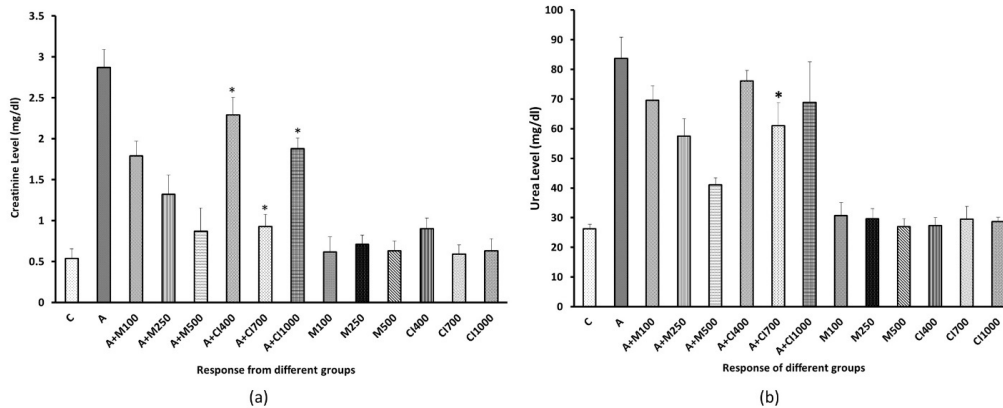


Figure 3: a) Creatinine level of rats belonging to 14 groups throughout receiving respective treatments b) urea level of rats belonging to 14 groups throughout receiving respective treatments. Values were expressed as mean \pm SD (n = 10/group). *p< 0.05 indicate significant difference from the disease group (C= control group, A=alloxan-treated group, M= metformin, A + M =alloxan + metformin, A +CI= alloxan + C. indica, and CI= C. indica).

In the experiment, creatinine level increased greatly in group Calloxan treated rats. Creatinine level was significantly low in group A + CI400, A + CI700 and A + CI1000 where alloxan and *Coccinia* extract was administered together. However, the creatinine level was much lower in the alloxan induced metformin treated rats in group A + M100, A + M250 and A + M500 than the alloxan induced extract treated rats of group A + CI400, A + CI700 and A + CI1000. However, in alloxan induced diabetic rats only medium dose at A + CI700 exhibited significantly low urea level but metformin in alloxan reduced rats of group A + M100, A + M250, A + M500 exhibited low urea level in a dose dependent manner. The results of kidney Function test can be observed in figure 3.

Liver Functioning Test: SGPT and SGUT level of 14 groups of rodents at the end of our study is demonstrated in the following graph.

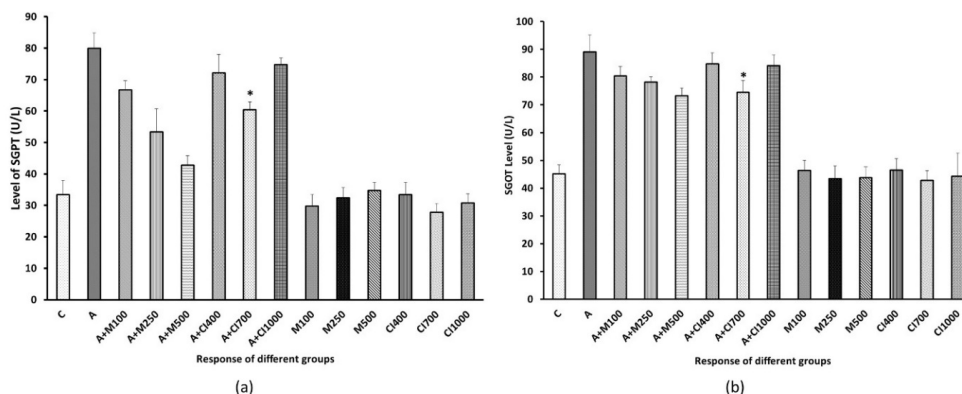


Figure 4: a) SGPT level of rats belonging to 14 groups throughout receiving respective treatments b) SGUT level of rats belonging to 14 groups throughout receiving respective treatments. Values were expressed as mean \pm SD (n = 10/group). *p < 0.05 indicate significant difference from the disease group (C= control group, A=alloxan-treated group, M= metformin, A + M =alloxan + metformin, A +CI= alloxan + C. indica, and CI= C. indica).

According to figure 04, *Coccinia* extract in medium dose in alloxan induced diabetic rats significantly decreased SGPT and SGUT level in alloxan induced Diabetic rats of group A + CI700 by inhibiting the action of alloxan which increases SGUT and SGPT level as seen in the positive control group. The non-alloxan induced group M100 to CI1000 as serial mentioned in graph singly treated with metformin and plant extract also kept the SGPT and SGUT level same as the control group.

Lipid Profile Test: Cholesterol, HDL, LDL and triglyceride level of 14 groups of rodents after our study is illustrated in the following graph.

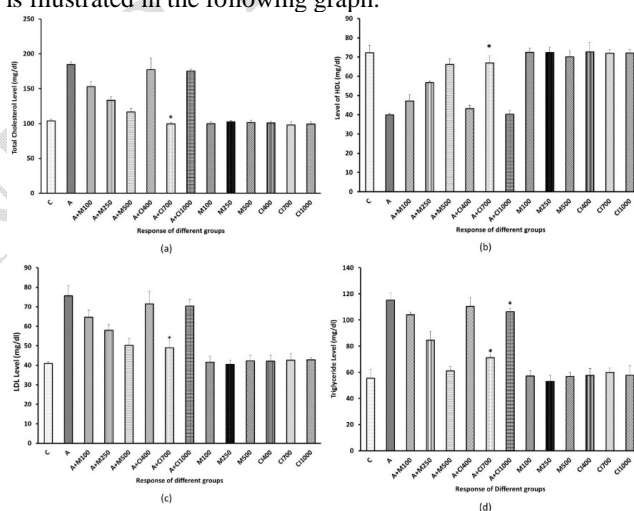


Figure 5: a) Cholesterol level of rats belonging to 14 groups throughout receiving respective treatments, b) HDL level of rats belonging to 14 groups throughout receiving respective treatments, c) LDL level of rats belonging to 14 groups throughout receiving respective

treatments d) Triglyceride level of rats belonging to 14 groups throughout receiving respective treatments. Values were expressed as mean \pm SD (n = 10/group). *p < 0.05 indicate significant difference from the disease group (C = control group, A = alloxan-treated group, M = metformin, A + M = alloxan + metformin, A + CI = alloxan + C. indica, and CI = C. indica)

Our study exhibited positive test in lipid profile function test. The alloxan combined with extract in medium dose in group A + CI700 reduced cholesterol, LDL and triglyceride level significantly but increased the HDL level significantly as shown in figure 05. However, extract in high dose in group A + CI1000 also decreased triglyceride level significantly.

Another 12-day study conducted on 30 male Wistar albino rats exhibited similar effects of lowering lipid and glucose plasma level where the rats were insulin resistant due to glucocorticoid administration.^[12]

Conclusion: Interpreting our results, it can be easily said that the plant can play a vital role in diabetic amelioration. However, the low dose food intake by rats of the high dose treated group indicates that the bitter taste of the extract is difficult to take without appropriate taste masking. So it can be inferred that more vigorous investigation and the precise isolation of anti-diabetic constituents from extract can enhance the potentiality of *Coccinia indica* becoming a part of the diabetic management system.

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