

In vivo assessment of antidiabetic potential and mapping of pharmacological properties of ethanolic extract of leaves of *Coccinia grandis* on alloxan-induced diabetic rats

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Abstract

Diabetes is a metabolic disease and plant derived products are used to combat this deadly disease. Plant is a diverse source of numerous therapeutic compounds which can be used to ameliorate diabetes. Leaves of *Coccinia grandis* (L.) (Family: Cucurbitaceae) is one of the leafy vegetable that is used for this purpose traditionally. It has been used against diabetes for a very long time. Our aims were to identify the hypoglycemic effect of extract of leaves of *Coccinia grandis* as well as to determine its safety profile so that we could use the plant material to improve the diabetic condition. Diabetes was induced in rats by intraperitoneal injection of alloxan at a dose of 150mg/Kg bodyweight and ethanolic extract of leaves of *Coccinia grandis* was fed to the rats at a dose of 750mg/kg. We measured blood glucose level, and safety profile by measuring SGOT, SGPT and creatinine level on diabetic and non-diabetic rats before and after administration of the extract. After measuring blood glucose level, it was found that the hypoglycemic efficacy was comparable to that of metformin ($p > 0.05$) which was given at a dose of 500 mg/kg. Safety profile were investigated by checking SGOT, SGPT and creatinine level. It was seen that both metformin and leaf extract of *Coccinia grandis* improved the pathological condition induced by diabetes. Furthermore, in healthy individual rats both metformin and leaf extract of *Coccinia grandis* did not significantly alter the normal physiological state. It might, therefore, be inferred that the extract of leaves of *Coccinia grandis* could be used as a good alternative therapy to treat diabetes.

Key words: *Coccinia grandis* (L.), Diabetes, Alloxan, Rat, Safety profile, SGOT, SGPT and Creatinine, Alternative therapy.

Introduction

Diabetes mellitus is a metabolic disorder impacting millions of patients globally. It is marked by absolute or relative inadequacies in insulin secretion or chronic hyperglycemia. It also occurs from dysfunctioning of carbohydrate, protein and lipid metabolisms [1]. World Health Organization (WHO) estimates that, 422 million adults over 18 years were living with diabetes in 2014 around the globe. Between 1980 and 2014, the number of people with diabetes has considerably raised approximately 4 times [2].

Coccinia grandis (L.) is an edible plant. It grows abundantly in Indian subcontinent and is a climbing perennial herb, growing throughout the South East Asia. It is broadly used in traditional medicament of diabetes [3]. Cucurbitaceae consists of 115 genera with 960 species, growing primarily in tropical and subtropical regions [4]. Prior to the invention of insulin in 1922, the medicaments for diabetes mellitus relied heavily on the herbal prepared from edible medicinal plants [5].

The leaf extract of *C. grandis* has been frequently used as an adjuvant therapy in Ayurvedic medicine for the treatment of diabetes mellitus [6]. Further therapeutic effects of leaves of the plant are anti-inflammatory and hepatoprotective [7, 8]. There is also a report of the acute hypoglycemic activity of the ethanolic extract of *C. grandis* in normoglycemic diabetic rats [9]. *Coccinia grandis* also has established properties like the effect of methanolic leaf extract on liver enzymes and lipid profile that has been experimented in streptozotocin induced diabetic rats [10]. Its pharmacological properties include antibacterial [11- 13], antimalarial [14-15], anthelmintic [16], anticancer [17-18], analgesic [19], antiulcerant [20-21], antitussive [22], antidyslipidemic [23], mutagenic effect [24] and alpha-amylase inhibition [25]. The modern drugs used in diabetes management is heavily expensive, and also burden and inaccessible for mass population. In our study design, we presented that *Coccinia grandis* can be used in accessible price that can potentially reduce the cost of conventional drugs. We expect that in future, we would be able to isolate the active component and use for treatment of diabetes.

Materials and Methods

Chemicals

The Active Pharmaceutical Ingredient (API) of metformin, an anti-diabetic drug, was obtained from Incepta Pharmaceuticals limited, Savar, Dhaka, Bangladesh and the leaves of the plant, *Coccinia grandis* was collected from Mirpur Botanical Garden, Dhaka, Bangladesh. Alloxan was purchased from Sigma Aldrich, Germany. SGOT, SGPT and Creatinine measurement kits were brought from Plasmatic Laboratory Product Ltd. Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer originated from Medigroup Asia limited, Cambodia) was used to measure the biochemical parameters and glucometer Alere GI of Alere Inc, USA was purchased from Shahbag, Dhaka, Bangladesh.

Extraction procedure

At first, the leaves were dried in sunlight for 3 days. Afterwards the dried leaves were crushed into powder and then powdered leaves were soaked in ethanol for 96 hours with occasional vigorous shaking. After the end of soaking, the extract was filtered and the filtered liquid was taken for the next step. The extracted solution was taken to the rotary evaporator machine to concentrate it. Then the dried extract was collected carefully [26].

Experimental design and Animal Handling

Healthy adult male Wistar rats (125-140gm) were collected from the Department of Pharmacy of Jahangirnagar University, Savar, Dhaka, Bangladesh and kept under controlled temperature (25°C) and 12±1h light/dark cycle at the Institute of Nutrition & Food Science, University of Dhaka. The animals were fed with standard pellet diet and water *ad libitum*. Before initiating the study the rats were kept there for acclimatization. After that body weight of each rat was measured and animals were divided into 6 groups where an even distribution of rodent as per their body weight has been taken place and each group contained 5 rats.

Group 1: Normal control.

Group 2: Alloxan induced control.

Group 3: Alloxan induced animals receiving metformin 500mg/kg of body weight [27].

Group 4: Alloxan induced animals receiving the extract of *Coccinia grandis* 750 mg/kg [28].

Group 5: Non-diabetic rats receiving 500 mg/kg metformin [27].

Group 6: Non diabetic rat receiving the extract of *Cocconia grandis* 750mg/kg [28].

At 1st two weeks the rats were treated with their respective specimen without inducing diabetes. Then alloxan, a chemical agent, was **injected** into all group 150mg/kg [29, 30] via intraperitoneal route except group one, five and six. Blood glucose levels of these rats were

checked to assure whether they are affected with diabetes after three days. The normal control group and alloxan **injected** control group were kept in untreated condition where the drug and extract treatment was initiated in animals of group 3, 5 and group 4, 6, respectively. The treatment was continued for thirty five days and the blood glucose level was checked once every week. The doses of drug and plant extracts were administered orally.

Statistical Analysis

The results of all study parameter belong to different groups were expressed as mean±SD. “One Way Anova Test” of SPSS 16” software was used to analyze intra-group and inter-group difference in results to find the statistical significance. Here statistical significance level was set at a ‘p’ value of $p>0.05$. The intra-group difference, in terms of results was considered statistically significant when the p value was found 0.05.

Results

Change in bod weights

Body weights of rats were measured initially and again prior to sacrifice. The differences between the changes in weight were taken into consideration. It has been observed that in the body weights of rats belonged to group 1, 4 and 6 were increased but decreased in group 2, 3 and 5. In control group, the bodyweight was increased by 7.5%; in alloxan induced diabetes group, bodyweight decreased by 5.8%; in metformin plus alloxan group, the bodyweight was decreased by 4.8%; in Coccinia grandis plus alloxan group, the bodyweight was increased by 9.8%; in only metformin group, bodyweight reduced to 3.4% and in only coccinia grandis group, bodyweight raised to 7.04%, respectively. The results are shown in Figure 1.

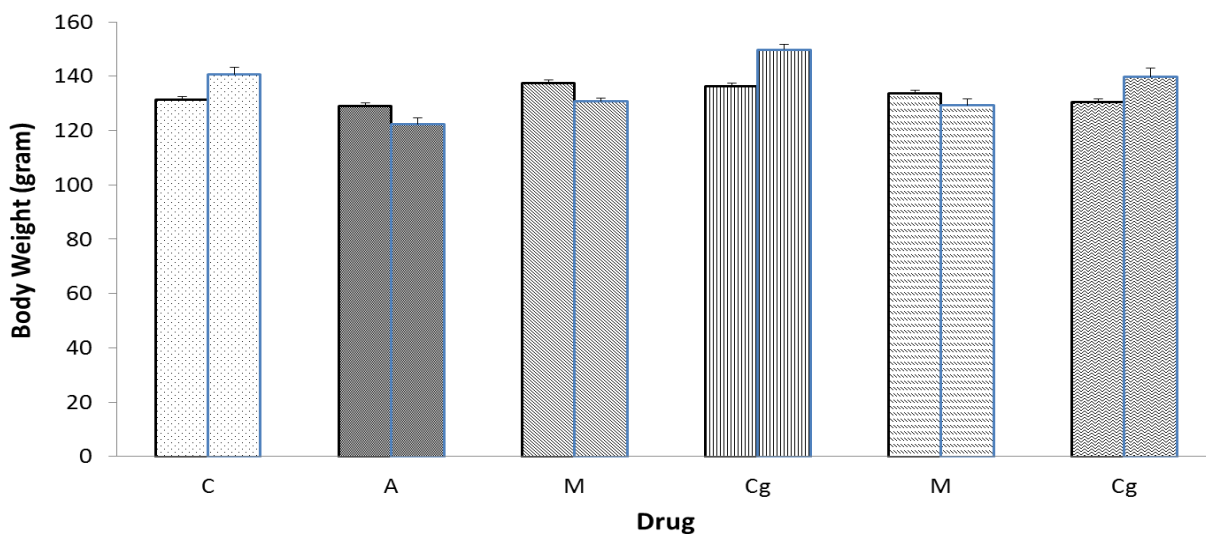


Figure 1: Comparison between the average body weight (mean±standard deviation) of rats belong to 6 groups at day one and day thirtyfive just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Cg=Alloxan+Coccinia grandis, M=Metformin, Cg=Coccinia grandis.

Change in blood glucose level

Blood glucose levels of rats belonged to 6 groups were measured once in a week for 35 days. It has been observed that blood glucose level of rats belonged to control group was 3.8 ± 0.32 mmol/L in day one and in day 35 it was 3.07 ± 0.24 ; in group 2, the alloxan control group, blood glucose level was observed 3.26 ± 0.25 mmol/L in day one and after administration of alloxan it became 19.36 ± 1.2 and 23.3 ± 1.37 mmol/L in day 35. In group 3 (alloxan+metformin) the blood glucose level was observed 3.53 ± 0.383 mmol/L in day one which was increased to 21.17 ± 1.3 mmol/L after administration of alloxan and was reduced to 6.1 ± 0.21 mmol/L at day 35 as metformin was induced. Similarly in group 4 the (Alloxan+Coccinia grandis) blood glucose level was initially 3.35 ± 0.31 mmol/L and alloxan made it 18.465 ± 0.47 mmol/L and after treatment with *Coccinia grandis*, it became 7.33 ± 0.50 mmol/L. In group 5 and 6 (metformin, *Coccinia grandis*) blood glucose levels were 3.7 ± 0.32 mmol/L and 3.65 ± 0.36 mmol/L, respectively, which became 2.93 ± 0.24 mmol/L and 3.53 ± 0.34 mmol/L in day 35. The results are shown in Figure 2.

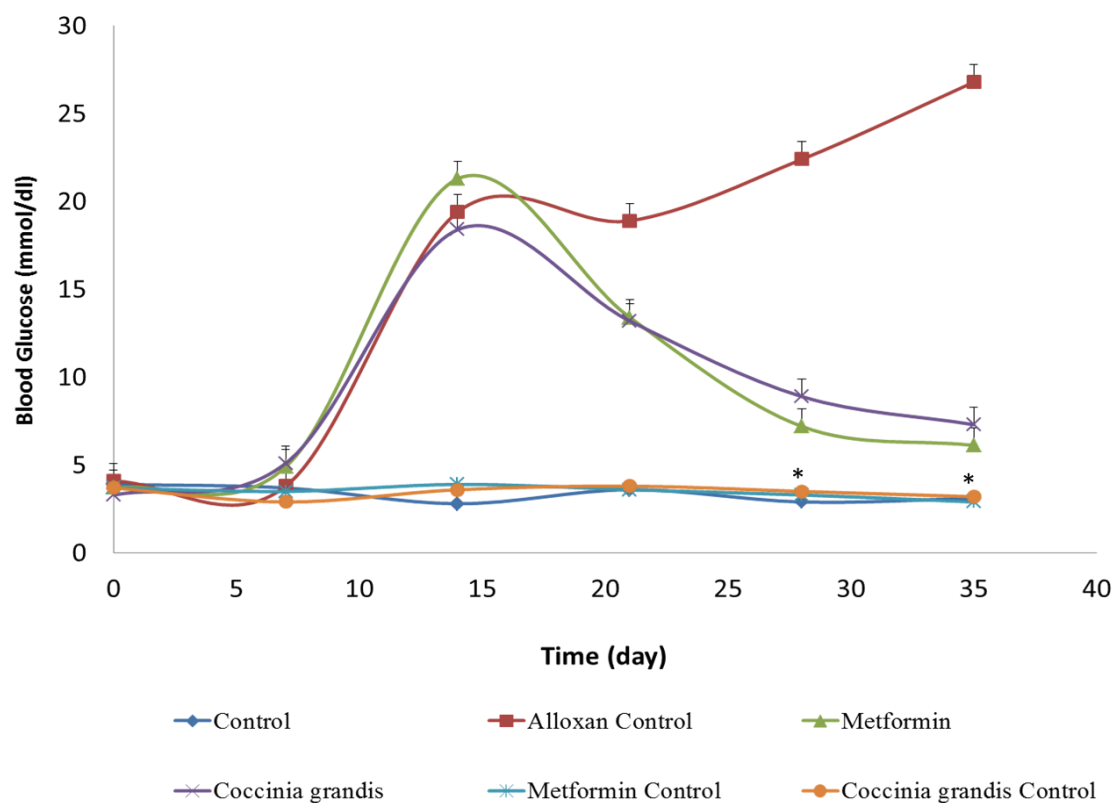


Figure 2: Blood glucose level of six groups from day zero to day thirty five. The data were expressed as mean± standard deviation. * Expresses the significant change.

Safety Profile Study (Liver function test)

The assessment of SGOT and SGPT levels were done after sacrificing to assess the function of liver as a safety profile test. The SGOT level of group 1 was found to be 46.2 ± 0.64 U/L and in group 2 it was observed 82.4 ± 1.57 U/L. In group 3 and 4 the observed SGOT level was 64.8 ± 2.82 U/L and 67.1 ± 4.09 U/L, respectively. SGOT level was observed to be 46.1 ± 1.04 U/L in group 5 and 43.9 ± 1.18 in group 6. The results of SGOT levels are shown in Figure 3.

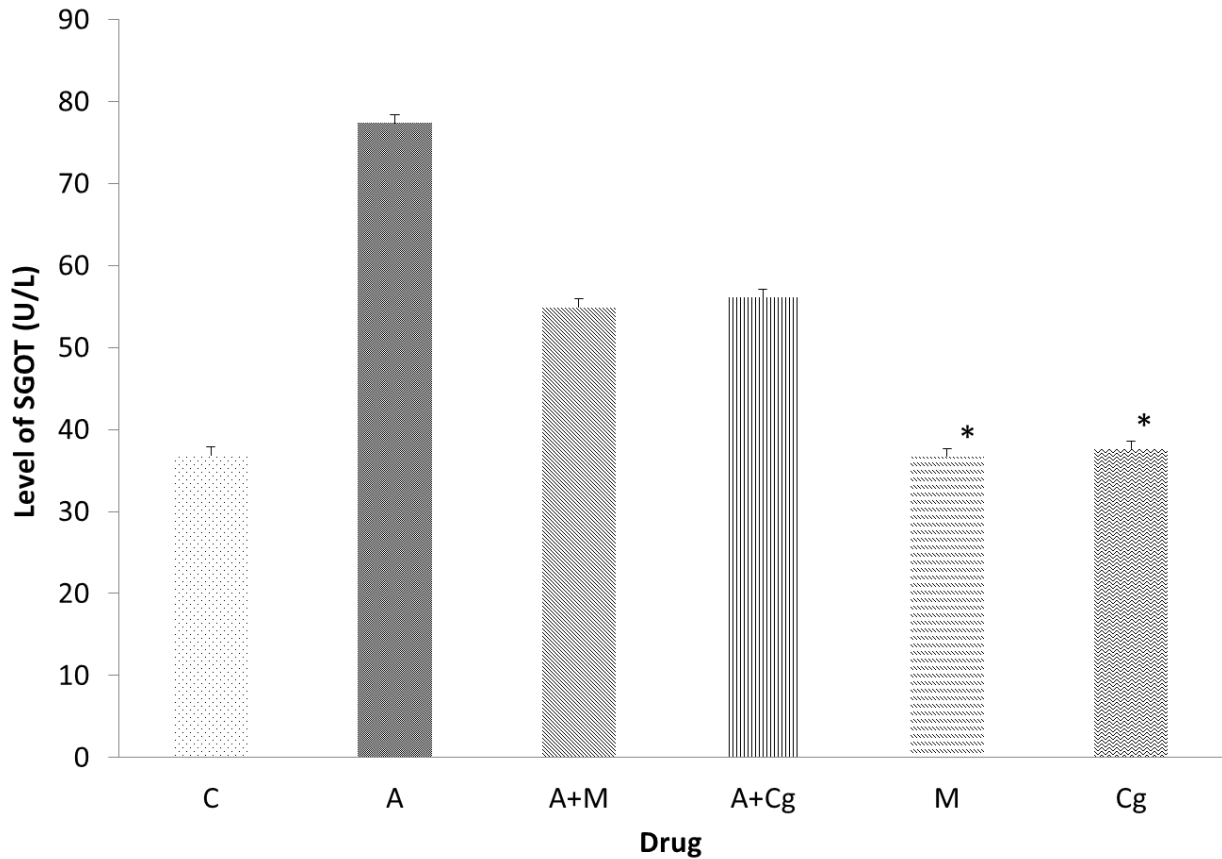


Figure 3: Comparison of SGOT level (U/L) of rats, of 6 groups at day thirty five after sacrifice. * Expressing the significant change. C=Control, A=Alloxan, A+M=Alloxan+Metformin, A+Cg=Alloxan+ Coccinia grandis, M=Metformin, Cg=Coccinia grandis.

Similarly in group 1 our inspected SGPT level was 36.9 ± 0.89 U/L and in group 2 which was noted as 77 ± 4.05 U/L. SGPT level of rats belonged to group 3 was found to be 54.9 ± 1.18 U/L and in group 4 it was 56.1 ± 0.52 U/L. As per our observation group 5 showed SGPT level of 36.5 ± 0.76 U/L and 37.1 ± 1.56 U/L in group 6. The results of SGPT levels are shown in Figure 4.

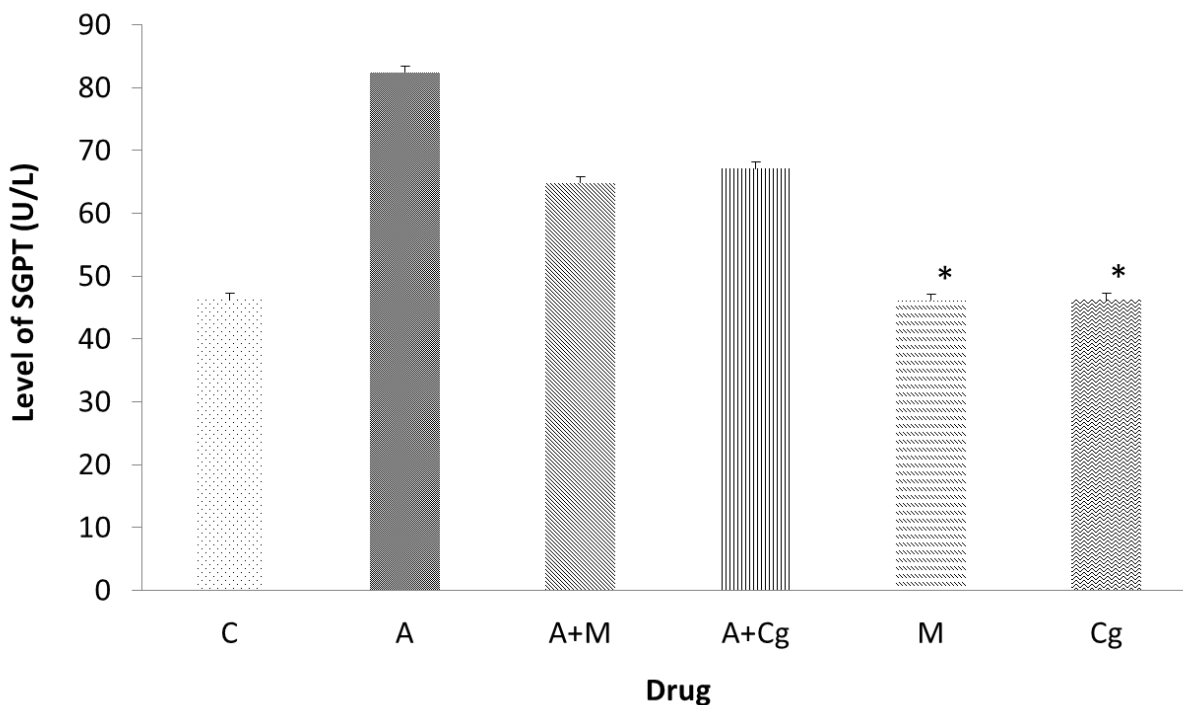


Figure 4: Comparison of SGPT level (U/L) of rats, belonged to 6 groups at day thirty five after sacrifice. * Expresses the significant change. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Cg=Alloxan+ Coccinia grandis, M=Metformin, Cg=Coccinia grandis.

Safety Profile Study (Kidney functioning Test)

Creatinine levels of rats were measured to assess whether the kidneys were functioning properly or not. Creatinine level was 0.8 ± 0.14 mg/dl in group 1 and 2.1 ± 0.23 mg/dl in group 2. In group 3 it was 1.1 ± 0.21 mg/dl and 1.2 ± 0.30 mg/dl in group 4. The creatinine level was 0.8 ± 0.11 mg/dl and 0.7 ± 0.16 mg/dl in group 5 and group 6, respectively. The results are shown in Figure 5.

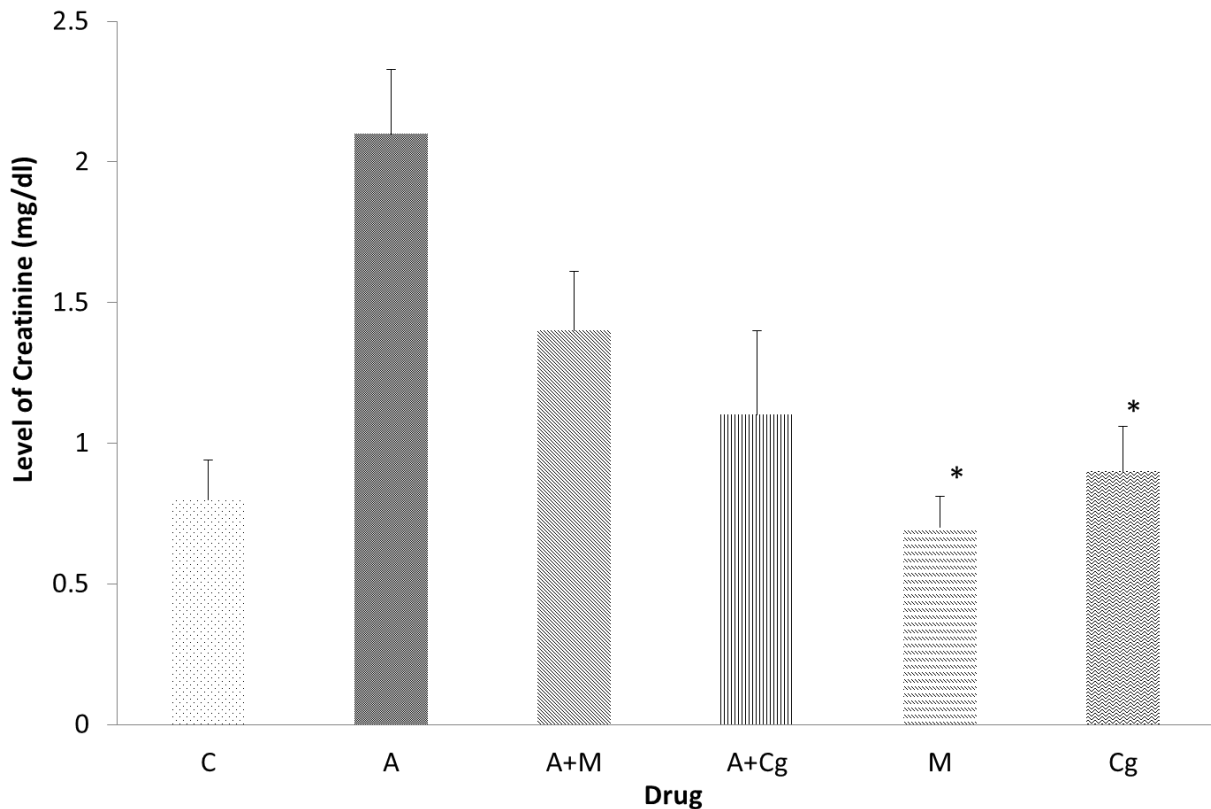


Figure 5: Comparison of Creatinine level (mg/dl) of rats, belong to 6 groups at day thirty five after sacrifice. * Expresses the significant change. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Cg=Alloxan+Coccinia grandis, M=Metformin, Cg=Coccinia grandis.

Discussion

In our study, we found that the body weight of rats belonged to group I (Control group), was increased. Whereas, in rats belonged to diabetic control and metformin-treated group, we observed a reduction of weight even though they were fed identically as control group. Here, in case of type 1 diabetes weight reduction is a physical phenomenon and metformin as a drug could not resist the reduction in body weight. Apart from that, in extract treated rat group the weight was increased but not like the control group. It can, therefore, be said that the plant extract can significantly nullify one symptom of type 1 diabetes. The blood glucose levels of group 1 rats were detected to be normal. In diabetic controlled group, due to destruction of beta cells and untreated condition, the blood glucose level was higher than all other groups. In both

metformin and extract treated group, the elevated blood glucose level (for beta cell destruction) was decreased in the same pattern. However, the reduction of blood glucose in metformin treated group was little bit higher than that of extract treated group but it does not possess any statistical significance. The SGOT, SGPT and Creatinine levels of control group were lower than all the other groups, in contrast the alloxan control group showed highest level of SGOT, SGPT and creatinine level in comparison to other groups due to the destructive effect of alloxan. Both drug-treated and extract-treated groups showed better condition than the diabetic control group but worsen the condition than the control group. And between drug and extract treated group the drug-treated group was a little bit better condition, still no statistical significance was found ($P>0.05$). Additionally we observed that the SGOT, SGPT, Creatinine and blood glucose levels of normal healthy rat treated with *Coccinia grandis* and metformin was almost similar to control group which can be termed as a marker of safety. Previously it has been proved that metformin did not cause hypoglycemia [31] and for *Coccinia grandis* we observed the same result, the plant extract and metformin did not cause hypoglycemia in normal healthy rats belonged to Group 6. Additionally the comparison among the rats belonged to group 4 and group 6 showed statistical significance ($p<0.05$). Moreover, the SGOT, SGPT and Creatinine levels of rats belonged to Group 6 did not show any statistical significance when compared with rats belonged to Group 1. These interpretations could be evidenced that the elevated level of SGOT, SGPT and Creatinine in Group 4 considering Group 1 and group 6 was due to the destructive effect of alloxan not for the plant extract itself. Furthermore, it can also be said that further modification and isolation of therapeutic compound of *Coccinia grandis* may provide us better effect than metformin. As there is no statistical significance in the difference of blood glucose lowering effect of Group 3 and group 4 as well as in SGOT, SGPT and Creatinine levels. But metformin itself is a single API which was given in 500 mg/kg body weight, in contrast the plant extract was given at a dose of 750 mg/kg body weight which contains numerous compounds and hence naturally its anti-diabetic effects will be lower than that of metformin.

Conclusion

From the above results, it may be concluded that the *C. grandis* leaf extracts provide similar but slightly lower effect than metformin with null statistical significance. Furthermore, in diabetic rats it improved the conditions of pathological parameters like SGOT, SGPT and Creatinine along with imparting anti-diabetic effect. Additionally these parameters are found unchanged

when non-diabetic rats were fed with *C. grandis* with similar dose. We, thus, conclude that these herbal remedy can be incorporated for disease management of type I diabetes mellitus.

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Conflict of Interest: None

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Authors’ contribution: This work was carried out in collaboration among all authors. Authors AAC and MSA designed and wrote the research protocol. The authors ZI, MRT, AUF and TIT performed the tests equally and analyzed the data with the active co-operation of TTN, TSN, MZHG, and FAN. Authors JAC and SK helped to trim the data of the work and performed the statistical interpretation. Author MSA has taken care of the whole project during the research protocol. All authors meticulously read and approved the final manuscript.

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