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IN VIVO EVALUATION OF ANTIDIABETIC AND ANTIDEPRESSANT POTENTIAL AND SIDE EFFECT STUDY OF AQUEOUS EXTRACT OF LEAVES OF *CENTELLA ASIATICA* ON ALLOXAN-INDUCED DIABETIC RAT MODEL

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ABSTRACT

Diabetes is a metabolic disease and depression is a mental aliment plant derived merchandise are used to fight this both of disease, plant is a various supply of several therapeutic compounds which may be used to ameliorate diabetes & depression. Leaves of Centella asiatica is one of the leafy vegetables this is used for this cause traditionally. It has been used towards diabetes & depression for a very long time. Our aims have been to pick out the hypoglycemic & antidepressive effect of extract of leaves of Centella asiatica as well as to decide its protection profile so that we could use the plant material to enhance the diabetic & depressive situation. Diabetes changed into precipitated in rats with the aid of intraperitoneal injection of alloxan at a dose of 150 mg/Kg bodyweight and ethanolic extract of leaves of Centella asiatica changed into fed to the rats at a dose of 750 mg/kg. We measured blood glucose degree, and safety profile by means of measuring SGOT, SGPT and creatinine stage on diabetic and non-diabetic rats earlier than and after administration of the extract. After measuring blood glucose level, it turned into observed that the hypoglycemic efficacy was similar to that of metformin ($p > 0.05$) which become given at a dose of 500 mg/kg. Safety profile had been investigated by measuring the

SGPT, SGOT and Creatinine Level. Become visible that both metformin and leaf extract of or plant can improve the pathological condition situation brought about by using diabetes, sucrose performance test and tail suspension test check has also shown extensive resopnse. Furthermore, in wholesome individual rats both metformim and leaf extract of Centella asiatica did no longer substantially alter the everyday physiological state. It might, therefore, be inferred that the extract of leaves of Centella asiatica might be used as a good alternative remedy to treat each diabetes & depression.

KEYWORDS: *Centella asiatica*, Alloxan, Kidney functioning test, Liver functioning test, Alternative therapy.

INTRODUCTION

For a very long period of time, diabetes has been illutrated as one of the world's foremost endocrine disorder. The amount of population to be affected by this disorder by the year 2000 was assumed to be 171 million, by World Health Organization.[1] Diabetes cannot be entirely cured rather it must be kept under tight control.[2] This control might be achieved, by changing lifestyle, medications, diet, or a combination of all of these.[3] Many of the drugs currently in use, are highly cost and have adverse effects which, in tandem, makes diabetes management even more tough.[4] Hence, complementary and Alternative Medicines (CAM) have a large room to bring about improvements in the current practices of diabetes management. CAM is hastily growing popular throughout the world.[5] Moreover, CAM therapy is relatively cheaper than synthetic, patented drugs in the perspective of developing countries. A large segment of the population in these countries conventionally rely on CAMs for medicating a multitude of disorders including, diabetes.[6] However, evidences concerning efficacy of these therapies are still sparse and their mechanism of action is often not clear.[7] Centella asiatica is a broadly used conventional remedy in both Africa and India. The principal constituents of C. asiatica is the triterpenic fractions that conferred a broad range of defensive and therapeutic outcomes.[8] C. asiatica is also a precious source of Amino acid, Terpenoid, Phenols, Volatile Oils fatty oils, phytosterols, resin, Vitamin.[9,10]

C. asiatica imperts various pharmacological actions such as, anti-inflammatory activity, antioxidant activity, anticancer activity, antiulcer activity, cardioprotective activity, hepatoprotective activity, wound healing, antimicrobial activity, neuroprotective, anti-hyperglycemic and antidepressant activity effect.[11-21]

The water extract of the whole plant is used by conventional healers in Tanzania for the management of both Type I and Type 2 diabetes. Studies have found C. asiatica possessing important hypoglycemic activity in glucose tolerance test in rabbits. It was also detected not to cause hypoglycemia in fasting rabbits compared to the standard tolbutamide.[22] The ethanolic extract demostrated an aggrandized glycogen content in the liver, comparable to the glibenclamide standard . Additionally, the extract demontrated lowered serum cholesterol and total lipid level.[23] Methanolic extract was determined to be more effective than ethanolic extract in managing to lower blood glucose. However, many aspects of basic mechanism of action of C. asiatica still remain unclarified to date. The aim of the current study is to depict a comprehensive portrayal of effects C. asiatica on sucrose breakdown and glucose absorption, insulin release, and intestinal enzyme functions.

MATERIALS AND METHODS

Chemicals

Metformin APIg, was collected from Square Pharmaceuticals limited, Dhaka, Bangladesh, and the plant more specifically, *Centella asiatica* was collected from the University garden Garden, Alloxan was bought from Sigma Aldrich, Germany.

Creatinine, SGPT, and SGOT measuring kits were collected from Plasmatic Laboratory Product Ltd. Humalyzer 3000 (Semi-Automated Clinical Chemistry Analyzer originated from Medigroup Asia limited, Combodia) was applied to estimate the biochemical parameters and glucometer Alere GI of Alere Inc, USA was obtained from Mirpur, Dhaka, Bangladesh.

Extraction procedure

Preparation of plant extract and decoction preparation: Fresh leaves of C. Asiatica were properly washed using just after obtaining and cut down into several pieces, then was made dry in air. Next, the dried leaves were crushed into powder. The obtained powder was then soaked in 2.5 liter of water for 10 min allowing the decoction to stand for 45 minutes then was filtered using Whatman no.1 filter paper. After that, the filtrate was concentrated under decreased pressure at the temperature underneath 50°C employing a rotatory evaporator. Then, the extracts were put in glass Petri-dishes to make it dry in air dry for maximum evaporation of the solvent.[24]

Experimental design and Animal Handling

Healthy adult Wistar albino male rats were collected from the Department of Nutrition and Food University of Dhaka, Dhaka, Bangladesh, and were maintained under 12±1h light/dark cycle controlled temperature (25◦C) and at the Institute of Nutrition & Food Science, University of Dhaka. The animals were fed with standard pellet diet and water *ad libitum.* Before launching the research the rats were kept there for acclimatization. After that body weight of each rat was marked 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 .

Group 4: Alloxan induced animals receiving the extract of *Centella asiatica* 800 mg/kg .

Group 5: Non-diabetic rats receiving 500 mg/kg metformin

Group 6: Non-diabetic rat receiving the extract of *Centella asiatica* 800 mg/kg.

At 1st weeks, the rats were administered with their food and water without inducing diabetes. Then alloxan, a chemical agent, was injected into all group 150mg/kg[25] via intraperitoneal route except group in all the rats belonged to group 2,3 and 4. Blood glucose levels of these rats were examined to assure whether they are affected with diabetes after three days. The normal control group and alloxan injected control group were kept in untreated conditions where the drug and extract treatment was initiated in animals of groups 3, 5, and group 4, 6, respectively. The treatment was continued for forty-two days and the blood glucose level was monitored once in a week. The doses were applied orally.

Statistical Analysis

The findings of all research parameters belong to several groups were represented as mean±SD. "One Way Anova Test'' of SPSS 16" software was applied to investigate the intra-group and inter-group differences in results to find the statistical significance. Here statistical significance level was set at a 'p' value of $p>0.05$. The intra-group diversity, in terms of results, was considered statistically significant when the p-value was obtained 0.05.

RESULTS

Change in bod weights

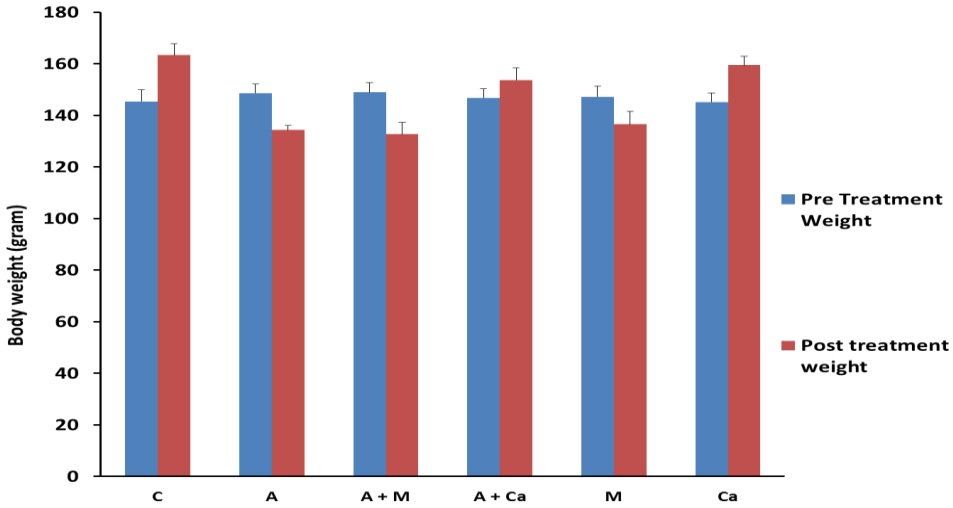

Figure 1: Comparison between the average body weight (mean±standard deviation) of rats.

belong to 6 groups at day onre and day forty two just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Ca=Alloxan+ *Centella asiatica*, M=Metformin, Ca= *Centella asiatica*

Change in blood glucose level

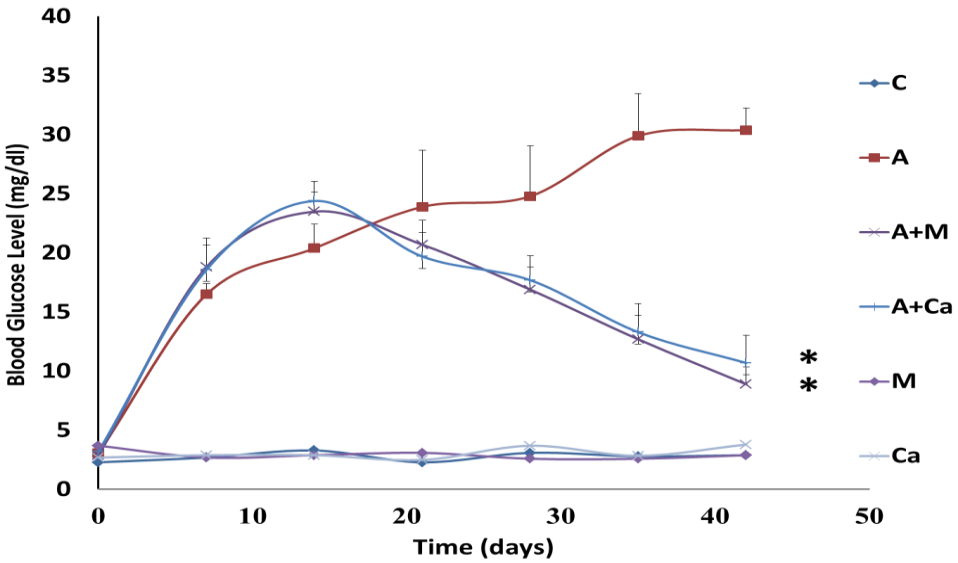

Figure 2: Blood glucose level of six groups from day zero to day forty two. The data were.

expressed as mean± standard deviation. * Expresses the significant change.

Safety Profile Study (Liver function test)

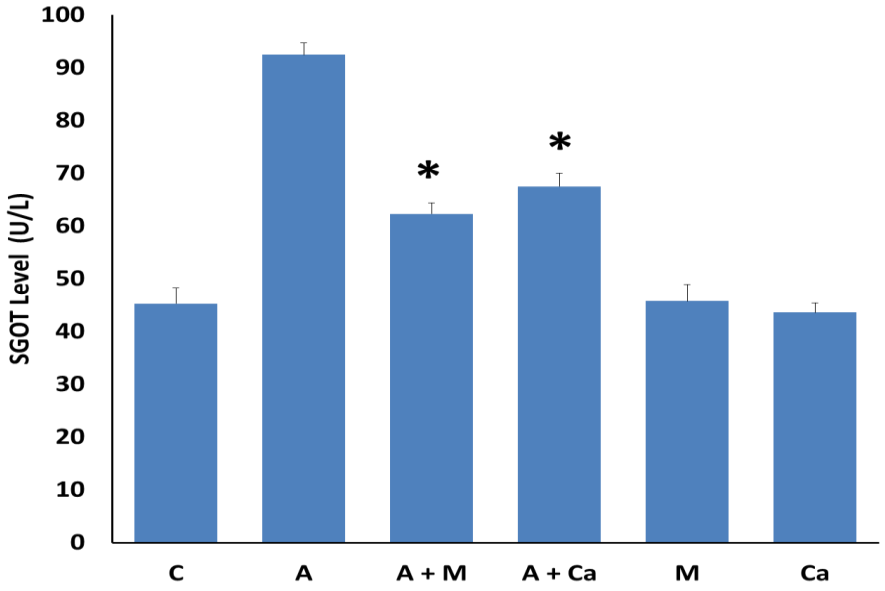

Figure 3: Comparison of SGOT level (U/L) of rats, of 6 groups at day forty two after sacrifice.

sacrific. * Expressing the significant change. at day onre and day forty two just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Ca=Alloxan+ *Centella asiatica*, M=Metformin, Ca= *Centella asiatica*

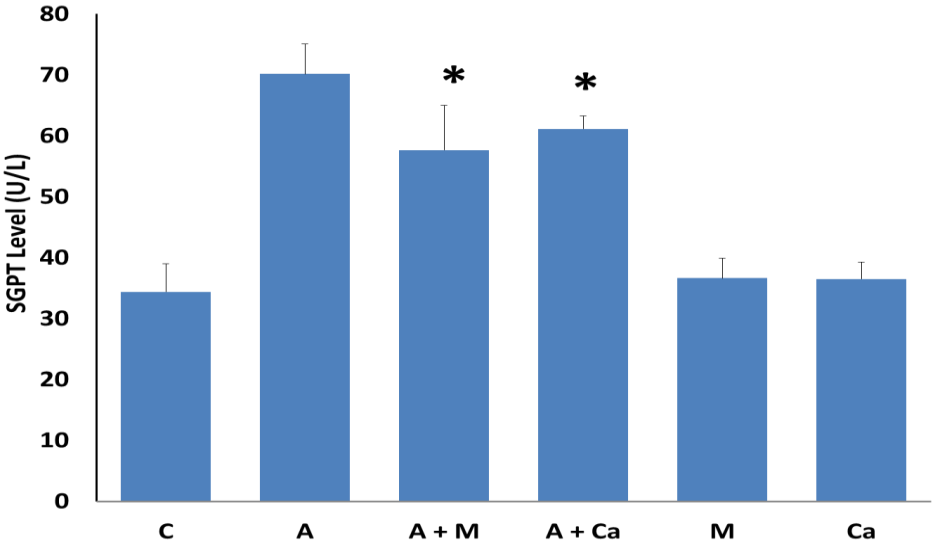

Figure 4: Comparison of SGPT level (U/L) of rats, of 6 groups at day forty two after sacrifice

sacrific. * Expressing the significant change. at day onre and day forty two just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Ca=Alloxan+ *Centella asiatica*, M=Metformin, Ca= *Centella asiatica*

Safety Profile Study (Kidney functioning Test)

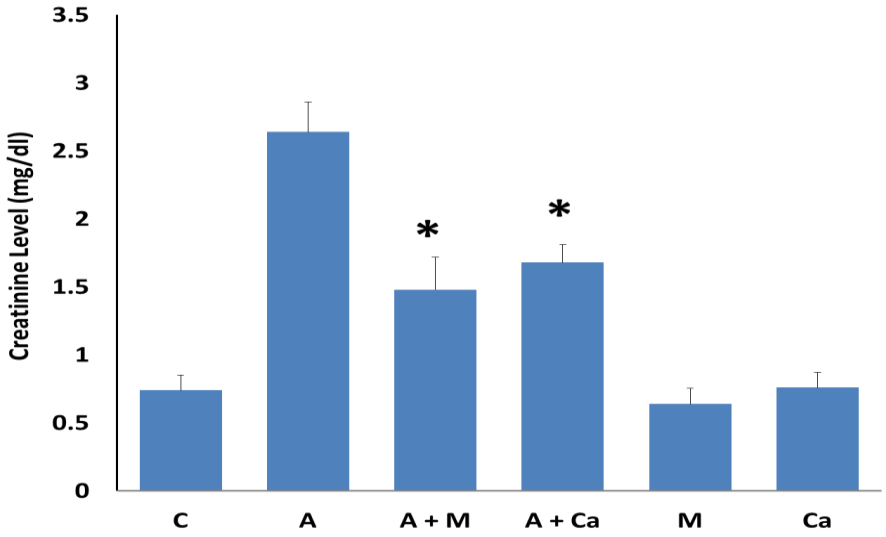

Figure 5: Comparison of Creatinine level (U/L) of rats, of 6 groups at day forty two after.

sacrifice sacrific. * Expressing the significant change. at day onre and day forty two just before sacrifice. C=Control, A=Alloxan, A+M= Alloxan+Metformin, A+Ca=Alloxan + *Centella asiatica*, M=Metformin, Ca= *Centella asiatica*.

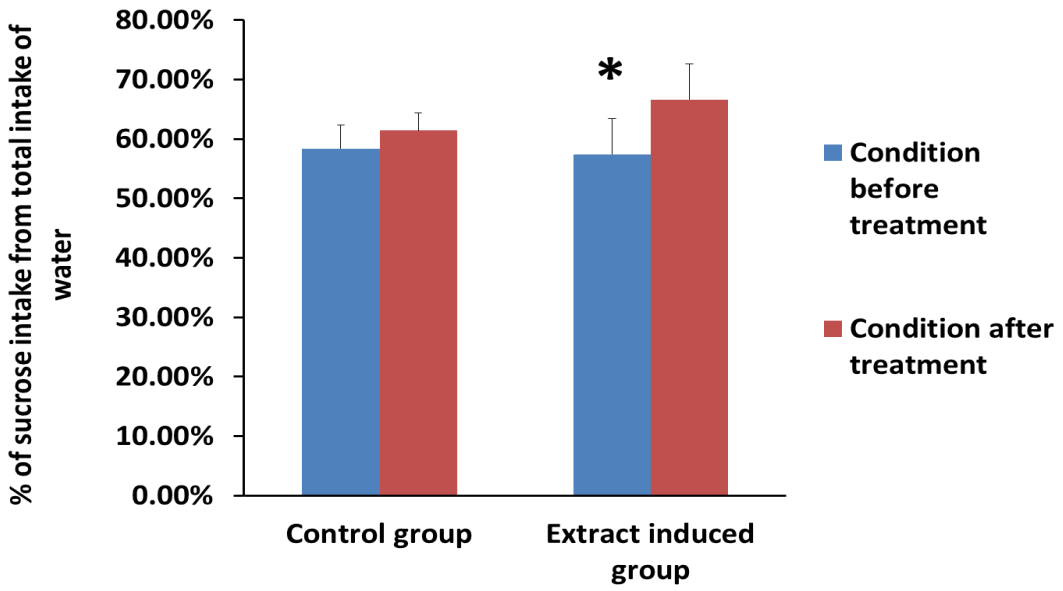

Figure 6: Comparison of response of exract treated group and control group to sucrose

Preference test at day one and at day forty two after sacrifice. * Expressing the significant difference between stressed & stressed group+ treatment . Again ● expressing significant difference between control group and stressed group

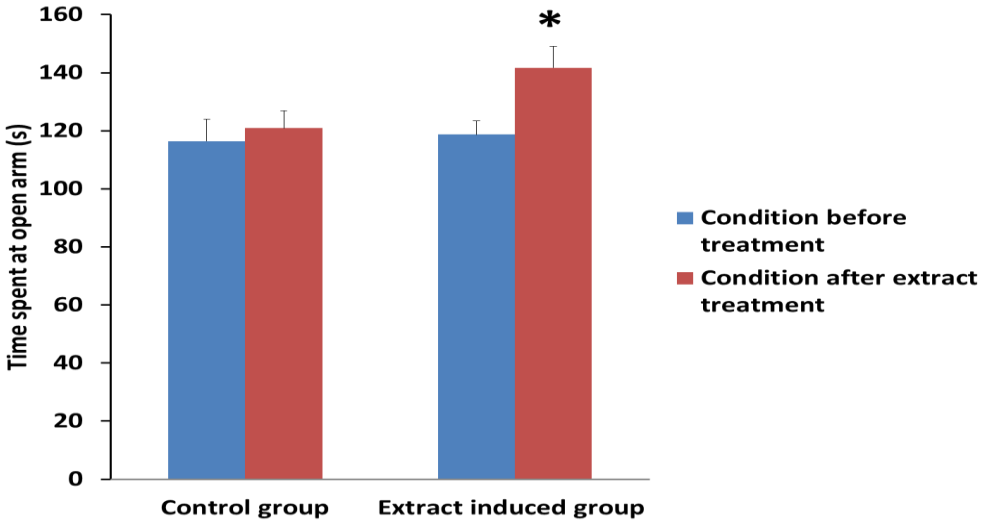

Figure 7: Comparison of response of exract treated group and control group to tail suspension test at day one and at day forty two after sacrifice. * Expressing the significant change.

DISCUSSION

It was found in our study that the body weight of rats which belonged to group I (controlled group) was increased. Whereas it was observed that the body weight of rats which belonged to diabetic control and metformin-treated group was decreased though they were fed same amount of food identically as control group. Weight reduction is a physical phenomenon in case of type I diabetes and body weight reduction could not be inhibited by Metformin as a drug. Besides that, the weight of extract-treated rat group was also increased but it was not same as the control group. So, we can say that one symptom of type 1 diabetes can be significantly nullified by the plant extract. In case of group 1 rats, blood glucose levels were detected to be normal. On the other hand due to destruction of beta cells and untreated condition, the blood glucose level was higher than all other groups in diabetic controlled group. Apart from that, it was seen that the elevated blood glucose level was decreased in the same pattern in both metformin and extract treated group for beta cell destruction. Howsoever, the reduction of blood glucose level was little bit higher in Metformin than extract-treated group but it does not reflect any statistical purport. The controlled group showed lower SGOT, SGPT and creatinine levels than all other groups, on the contrary highest level of SGOT, SGPT and creatinine levels were shown by the alloxan control group in comparison to other group due to destructive effect of alloxan. Moreover, it was seen that drug-treated and extract-treated both groups showed a better condition than the diabetic control group but worsen the situation than the control group. And the drug-treated group was

a little bit better condition than the extract treated group though any statistical significance was not found ($p>0.05$). Additionally, it was observed that the SGOT, SGPT, Creatinine and blood glucose levels of healthy rat was very nearly equivalent to control group when they were treated with *Centella asiatica* and metformin which can be termed as a marker of safety. Formerly, it was turned out that metformin was not responsible for hypoglycemia and we observed the similar outcome for *Centella asiatica,* it can be said that the plant extract and metformin did not cause hypoglycemia in normal healthy rats belonged to Group 6. Additionally, the parallelism within the rats belonged to group 6 and group 4 showed statistical significance ($p<0.05$). Furthermore, the SGOT, SGPT and Creatinine levels did not indicate any statistical significance in the rats belonged to Group 6 when it was compared with rats belonged to Group 1. By these interpretations, it could be asserted that the elevated level of SGOT, SGPT and Creatinine level in Group 4 considering Group 1 and group 6 was owing to the destructive effect of alloxan, not for the plant extract itself. In addition, we can say that further modification and isolation of therapeutic compound of *Centella asiatica* may provide us better effeicacy than metformin as there is no statistical significance in the difference of blood glucose, SGOT, SGPT and Creatinine lowering effect of Group 3 and group 4. Metformin was given in 500 mg/kg body weight which is a single API itself, on the other hand the plant extract which contains numerous compounds was given at a dose of 750 mg/kg body weight and consequently anti-diabetic effects of plant extract will be lower than that of metformin.

Apart from that it has also been observed that the plant extract can impart antidepressant effect. In sucrose preference test the percentage of intake of sucrose water was increased significantly in extract treated rat than that of negative control group. Consequently, in Elevated plus Meze test the time spent in open arm increased significantly in extract treated group. It confer that plant extract can provide both anti diabetic and antidepressant effect.

CONCLUSION

From the mentioned outcomes, it can easily be said that the leaf extract of *Centella asiatica impart* alike metformin however the effect is somewhat lower than that of metformin. But this difference does not bear any statistical significance, also it improved the pathological state like SGPT, SGOT, and creatinine level as like as the hypoglycemic effect. Moreover, the data received from healthy rats those who were fed the extract, were similar to the

negative control group. Hence we may infer that our plant can be applied in the control of diabetes mellitus type 1.

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Conflict of Interest: None.

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