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HEMATOBIOCHEMICAL EFFECTS OF TELAKUCHA (*Coccinia indica)* IN ALLOXAN INDUCED DIABETIC RATS

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A B S T R A C T

The study was undertaken to investigate the effects of Telakucha *(Coccinia indica)* on blood glucose, serum total cholesterol, hematological parameters and body weight in alloxan induced diabetic rats. In this experiment 45 rats were selected for the trial purpose. The rats were divided into 3 equal groups, each containing 15 individuals (n=15) as follows: normal control (C) group, diabetic control (DC) group and diabetic treatment (DT) group. Diabetes was induced in diabetic control (DC) and diabetic treatment (DT) group with alloxan @ 200mg/kg body weight intraperitonially. Then diabetic treatment group (DT) was treated with 10% aqueous extract of Telakucha @500 mg/kg body weight. After 42 days of treatment, Telakucha extract reduced the amount of blood glucose significantly *(P*<0.001) in the group DT compared to DC from 32.03 ± 0.25 to 11.17 ± 0.08 mmol/L. Total cholesterol (TC) was also decreased significantly *(P*<0.001) in group DT compared to group DC from 121.85±0.27 to 112.42±0.14 mg/dL. In hematological study, DT group showed significant *(P*<0.001) increase in erythrocytes count and Hb (g%) content and decrease *(P*<0.001) in total leukocyte count after 42 days of treatment in contrast with DC group. The body weight was also increased significantly (*P*<0.001) in DT group. Based on present research it can be concluded that Telakucha *(Coccinia indica)* can be used in the treatment of diabetes as an alternative to commercial medicine.

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INTRODUCTION

Inadequate production of insulin by the pancreas or the inability of the body to respond to the insulin can develop a metabolic disorder that is Diabetes Mellitus (DM). DM mostly affects humans as a major health problem and characterized by hyperglycemia with long term complications like retinopathy, nephropathy, neuropathy and macro vascular disease (Ota and Ulrih, 2017). Globally, 463 million people have diabetes and this number is projected to reach 578 million by 2030, and 700 million by 2045 (IDF, 2019). In Bangladesh, the number of detected diabetic patients reached 8.4 million along with the undetected diabetic patients almost 4.7 million (IDF, 2019). Oxidative stress due to chronic hyperglycemia during DM causes tissue and organ damage by increasing oxidation of carbohydrate, protein, lipid and DNA (Prasath and Subramanian, 2013; Yachamaneni and Dhanraj, 2017). Treatment of diabetes with modern synthetic drugs has series of adverse effects (Balaraman *et al*., 2010) and the cost is also increasing alarmingly in recent years (IDF, 2019); so, it is one of the crucial areas of investigation to find out new safer, easily available and more effective anti-hyperglycemic agents. *Coccinia indica*, known in Bangladesh as 'Telakucha', has been shown to possess hypoglycemic activity and it has significant role in the herbal treatment of diabetes (Ocvirk *et al.,* 2013). The plant extract proved to have beneficial hypoglycemic effect possibly by insulin secreting action or by influencing the enzymes engaged in glucose metabolism (Singh, 2011). Various phytoconstituents are found in *Coccinia indica* like cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol among them terpenoids are bleived to be responsible for antidiabetic activity (Deokate and Khadabadi, 2011). The objective of this study was to investigate the effects of *Coccinia indica* (Telakucha) leaf extract on blood glucose, serum total cholesterol and hematological parameters like total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin percentage (Hb%) in diabetic rats.

MATERIALS AND METHODS

This study was conducted in the Department of Anatomy, Histology and Physiology, Sher-e-Bangla Agricultural University, Dhaka to evaluate the efficacy of *Coccinia indica* (Telakucha) leaf extract in diabetic rats.

Collection and acclimatization of rats

Total forty five (45) healthy mixed albino rats were collected from Jahangirnagar University, Savar, Dhaka. For three experimental trials, all the rats were grouped into 3 groups each containing 15 rats. Each group was kept in separate cages and arranged in rows according to group. The animals were fed with pellet at a recommended dose of 100 g/kg body weight. Drinking water was supplied ad libitum. Before starting the experiment, all the rats were acclimatized to this environmental condition for a period of one week.

Experimental design

In this study, a total of 45 rats (15 normal rats and 30 alloxan induced diabetic rats) were used for each trial. The rats were divided into 3 groups each containing 15 individuals (n=15) as follows:
Group C: Normal control
Group DC: Diabetic Control (Alloxan)
Group DT: Diabetic Treatment (Alloxan + Telakucha treated)

Induction of diabetes in rats

Diabetes meliitus was induced in 2 groups of rats (DC and DT) with alloxan at the rate of 200 mg/ kg body weight intraperitoneally as a single dose (Ighodaro *et al.*, 2017).

Preparation and administration of Telakucha leaf extract (TLE)

Telakucha (*Coccinia indica*) leaves were collected from Sher-e-Bangla Agricultural University campus and dried by using freeze dry method and powdered with the help of mortar and pastel. From the powder 10% aqueous solution of telakucha (*Coccinia indica*) leaf extract was prepared followed by the procedure as that of (Singh, 2011) and group DT was treated with telakucha (*Coccinia indica*) leaf extract at the rate of 500 mg/ kg/ day for 42 days treatment period.

Determination of blood glucose (mmol/L)

Blood samples were collected from tail vein on 0, 21st and 42nd day of experiment and blood glucose was determined by using glucose oxidase-peroxidase reactive strips and a glucometer (UNI-CHECK®, Visgeneer, Taiwan).

Determination of total cholesterol (mg/dL)

Blood samples were collected from tail vein on 42nd day of experiment and blood cholesterol was determined by using a blood testing meter (EasyMate® GCU, Bioptik Technology Inc., Taiwan).

Collection of blood for hematological tests

On 42nd day of experiment, blood was collected directly from the heart with the sterile syringe and needle after anaesthetizing the animals with Xylazine HCl® @ 10mg/kg and Ketamine HCl® @ 80mg/kg body weight intramuscularly (Giroux *et al.*, 2016). About 1mL of blood from the syringe was taken in the vacuum tube containing anticoagulant (K3EDTA) for hematological studies.

Determination of hematological parameters

Total erythrocyte count (million/cumm)

Blood sample was diluted 1:100 with RBC diluting fluid with the help of RBC pipette. It was mixed properly and a drop was placed under the cover slip of the hemocytometer and red blood cells were allowed to settle for 3 minutes. The number of RBC present in 16x5 squares was counted under (45X) microscope (Dacie *et al.,* 1958 and D'Armour *et al*., 1965).

Total leukocyte count (thousand/cumm)

Blood sample was diluted 1:20 with WBC diluting fluid with the help of WBC pipette. It was mixed properly and a drop was placed under the cover slip of the hemocytometer and white blood cells were allowed to settle for 3 minutes. The number of WBC present in 16x4 squares was counted under (10X) microscope (Dacie *et al*., 1958 and D'Armour *et al.,* 1965).

Estimation of Hemoglobin (g%)

20 µl blood was taken in Sahli's Hemoglobinometer and diluted with 0.1 (N) HCl and color was matched with that of the standard one (Sood, 1999).

Determination of Body weight (g)

Body weights of the rats of all groups were recorded before treatment (on day 0) and during treatment period i.e. 7th, 14th, 21st, 28th, 35th and 42nd day by using weighing machine.

Statistical Analysis

All data were expressed as mean ± SEM (n=15) and differences among the groups of animals were compared using one-way ANOVA with post-hoc LSD and Duncan's test. Statistical significance was set at $P < 0.05$. Statistical analysis was performed using SPSS software version 25 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The study was carried out to evaluate the effects of *Coccinia indica* (Telakucha) on blood glucose, serum total cholesterol (TC), hematological parameters (TEC, TLC, Hb%) and body weight after 42 days of treatment in diabetic rats.

Effects on blood glucose level

The effects of Telakucha on blood glucose level to control diabetes in rats are presented in

Figure 1. In DT group, 10% aqueous solution of Telakucha leaf extract was significantly (P<0.001) found to reduce blood glucose levels to 16.18±0.16 and 11.12±0.08 mmol/L in 21st and 42nd day respectively comparing with the DC group. Shibib *et al.* (2012) reported the similar finding, in their study blood sample was collected and tested for once only just after 90 minutes of administration of Telakucha extract. Balaraman *et al*. (2010); Ramakrishnan *et al.* (2011) also found significant effects of Telakucha in lowering blood glucose but the treatment period of their study was shorter than that of present study. The level of blood glucose may be increased due to β cells destructions in diabetes (Bopanna *et al.* 1997). The plant extract showed to exert beneficial hypoglycemic effect possibly through an insulin secreting effect or through influence of enzymes involved in glucose metabolism (Singh, 2011), by depressing the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and by elevating both the red-cell and hepatic glucose-6-phosphate dehydrogenase (G6PDH) activities (Shibib *et al.,*2012).

Effects on serum total cholesterol

The effects of *Coccinia indica* (Telakucha) on serum total cholesterol (TC) are shown in Figure 2. At the end of the experimental period, it was found that, total cholesterol level was raised significantly (*P*<0.001) on diabetes induced rats (group DC) in comparison to control (C) group. Total cholesterol value decreased significantly (*P*<0.001) in Diabetic Treatment (DT) group by treating them with Telakucha in alloxan induced diabetic rats. Alloxan causes the production of excess fatty acid in plasma that enhances the liver conversion of home fatty acids into phospholipids and cholesterol (Rhoads *et. al*., 1976). Following the treatment with *Coccinia indica* extract, the serum total cholesterol level was significantly reduced (*P*<0.001). This result is similar with Balaraman *et. al*. (2010) who found that administration of *Coccinia indica* extracts tend to bring the level to near normal that might be due to the increase in the activity of lecithin cholesterol acyl transferase, which may contribute to the regulation of blood lipids.

Effects on hematological parameters

Effects on total erythrocyte count (TEC)

Effects of *Coccinia indica* (Telakucha) on total erythrocyte count (TEC) of diabetic rats are shown in Table 1. After induction of diabetes by alloxan TEC values were significantly (*P*<0.001) reduced in DC group to 5.31±0.08 million/cumm compared to control (C) group. On the other hand, TEC values were significantly (*P*<0.001) increased up to 9.21±0.06 million/cumm in DT group compared to DC group. Similar effects of *Coccinia* extract were found during the experiment of 'in vitro anticancer activity of *Coccinia'* that showed significant protective role to increase the RBC level (Bhattacharya *et al*., 2011).

Table 1. Effects of Telakucha on Hematological Parameters

Groups	Mean ± SEM		
	TEC (million/cumm)	TLC (thousand/cumm)	Hb (g%)
Control (C)	^{b}8.43±0.11	^{b}8.74±0.05	^{b}8.64±0.07
Diabetic control (DC)	^{c}5.31±0.08***	^{a}9.61±0.08***	^{c}6.47±0.08***
Diabetic Treatment (DT)	^{a}9.21±0.06***	^{c}8.44±0.06***	^{a}9.25±0.06***

Data are shown as mean±SEM (n = 15 samples per group).Values with different superscripts within a column differ significantly (*P*<0.05); *** =** Significant at 5 percent level (*P*<0.05); **** =** Significant at 1 percent level (*P*<0.01); ***** =** Significant at 0.1 percent level (*P*<0.001).

Effects on total leukocyte count (TLC)

Table 1 represents the effects of *Coccinia indica* (Telakucha) on total leukocyte count (TLC) in alloxan induced diabetic rats. The results indicated that after alloxan administration, TLC was significantly (P<0.001) increased in DC group compared to control group. After getting treatment, there was a significant (P<0.001) reduction of white blood cells in DT group compared to the DC group. Bhattacharya *et al*. (2011) also found that *Coccinia* extract can decrease the TLC level significantly (P<0.01) near the normal condition.

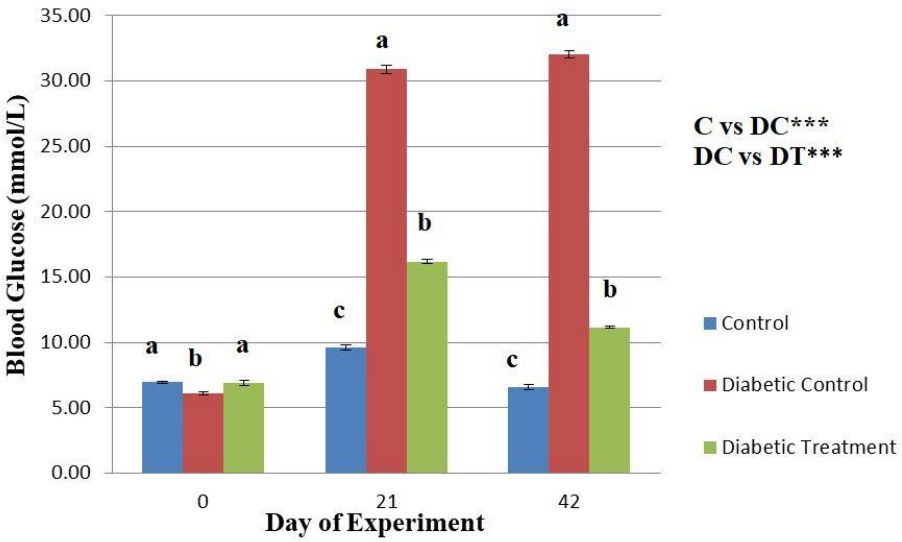

Figure 1. Effects of Telakucha on Blood Glucose (mmol/L)

Here, C= Control, DC= Diabetic Control, DT= Diabetic Treatment. Data are shown as mean±SEM (n = 15 samples per group).Values with different superscripts within a column differ significantly (P<0.05); * = Significant at 5 percent level (P<0.05); ** = Significant at 1 percent level (P<0.01); *** = Significant at 0.1 percent level (P<0.001)

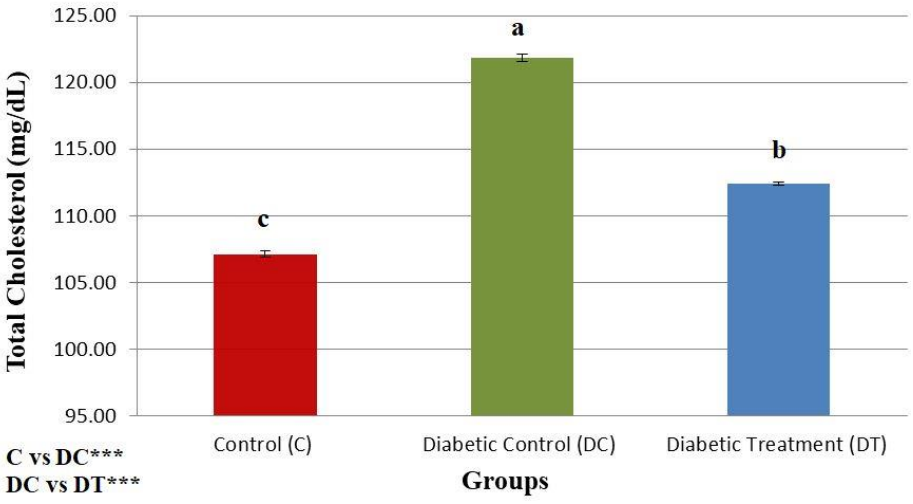

Figure 2. Effects of Telakucha on Total Cholesterol (mg/dL)

Data are shown as mean±SEM (n = 15 samples per group).Values with different superscripts within a column differ significantly (P<0.05); *** =** Significant at 5 percent level (P<0.05); **** =** Significant at 1 percent level (P<0.01); ***** =** Significant at 0.1 percent level (P<0.001). Here, C= Control, DC= Diabetic Control, DT= Diabetic Treatment

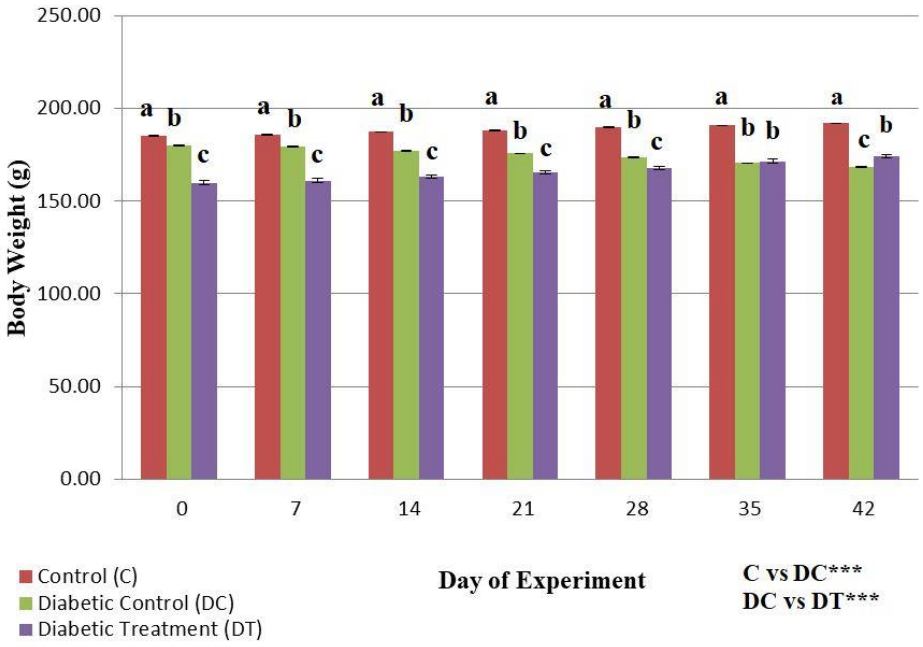

Figure 3. Effects of Telakucha on Body Weight (g)

Data are shown as mean±SEM (n = 15 samples per group).Values with different superscripts within a column differ significantly (*P*<0.05); * = Significant at 5 percent level (*P*<0.05); ** = Significant at 1 percent level (*P*<0.01); *** = Significant at 0.1 percent level (*P*<0.001). Here, C= Control, DC= Diabetic Control, DT= Diabetic Treatment

Effects on hemoglobin content

Table 1 shows the effect of *Coccinia indica* (Telakucha) on hemoglobin (g%) content in control and diabetic rats. In diabetic control (DC) group hemoglobin content was significantly (*P*<0.001) reduced to 6.47±0.08 g% after alloxan administration compared to control (C) group rats. After treating them with *Coccinia indica* (Telakucha), there was significant increase in hemoglobin content to 9.25±0.06 g% in diabetic treatment (DT) group. The present study is partially in agreement with findings of Venkateswaran and Pari (2002) and Bhattacharya *et al*. (2011) who found that *Coccinia indica* extract was able to increase hemoglobin significantly due to its protective nature.

Effects on body weight

Figure 3 shows the effects of administration of *Coccinia indica* (Telakucha) on body weight of alloxan induced diabetic rats. After induction of diabetes in rats, there was a significant (*P*<0.001) reduction in body weight of diabetic control (DC) group rats to 168.44±0.19g. After treating with *Coccinia indica* (Telakucha) a significant (*P*<0.001) increase in body weight was observed to 174.24±1.03g in diabetic treatment (DT) group compared to diabetic control (DC) group rats. Results of the present study partially support the findings of Venkateswaran and Pari (2002) who also observed significant increase in body weight after treatment with *Coccinia indica* extract in hyperglycemic animals, apparently due to its ability to reduce hyperglycemia. This may be due to some constituents of the *Coccinia indica* extract which may have mimicked or stimulated the actions of growth factors hence its ability to enhance the repair and regeneration of damaged pancreatic tissue. This position is strongly supported considering the fact that significant (*P*<0.05) increase in growth rate was obtained for rats receiving *Coccinia indica* extract treatment compared with those without extract treatment (Shibib *et al.*, 2012).

This study was conducted to elucidate the effects of Telakucha leaf extract on blood glucose, serum total cholesterol, hematological parameters (TEC, TLC, Hb) and body weight for a period of 42 days of treatment. Several studies have shown the significant effects of Telakucha on blood glucose, serum total cholesterol and body weight. But very few data have been found regarding hematological parameters (TEC, TLC, Hb) in diabetic rats. In the present research, attempts were made to investigate the protecting effects of Telakucha leaf extract on TEC, TLC and Hb content along with others parameter and it was found to be effective.

CONCLUSION

In this study, it was found that *Coccinia indica* (Telakucha) leaf extract has effective role in lowering blood glucose and total blood cholesterol level with regulatory effects on Hematological parameters like TEC, TLC and Hb in alloxan induced diabetic rats. To draw a definite conclusion in this regards it demands details study including histopathology and other biochemical parameters that indicate the conditions of vital organs like pancreas, liver and kidney in the treatment of diabetic rats with the supplementation of *Coccinia indica* (Telakucha).

COMPETING INTEREST

The authors declared there is no conflict of interest

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