

Protection from Arsenic Induced Hyperglycemia by a Traditional Plant - A Preliminary Study

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Abstract

Arsenic contamination in water is a major threat to human health and among the health hazards hyperglycemia is important. The current investigation was planned to find some remedial treatment of arsenic induced hyperglycemia by dietary supplementation of a traditional plant. This study was performed for a period of 30 days on adult male Wistar rats weighing 120-140 g. They were divided into three equal groups (n=6): Group Control (C), normal saline administered, Treatment Group 1 (T1), arsenic trioxide fed at the dose of 3 mg/kg/day for 30 days; and Treatment Group 2 (T2), arsenic trioxide at the dose of 3 mg/kg/day for 30 days along with powder of leaves of *Costus igneus* fed at the dose of 500 mg/kg/day for the last 15 days of arsenic treatment. The body weights and blood sugar levels were recorded on Days 1, 15 and 30. The animals were sacrificed on the 31st day and pancreas was collected for histological study of islets cells. The body weights of both the groups T1 and T2 decreased significantly (*p<0.05) after 15 days of arsenic treatment. A significant increase in body weight of T2 was found in the next 15 days of *Costus* supplementation in comparison to T1. The blood sugar levels of both T1 and T2 increased significantly (*p<0.05) after 15 days of treatment but it was reduced significantly in T2 after the next 15 days of *Costus* supplementation. The histology of the pancreatic islets cells in T1 illustrated that larger number of islet cells shrunken with lower number of cell counts in comparison to C. However, in T2, cell counts and morphology was restored significantly by *Costus* supplementation demonstrating that *Costus igneus* may be an effective agent in protection from arsenic induced hyperglycemia. From these preliminary findings further studies are warranted to elucidate the molecular mechanism of this plant's anti-hyperglycemic action.

Keywords: Arsenic; *Costus igneus*; Blood sugar; Hyperglycemia; Male rat

Introduction

India accounts for the largest number of people living below poverty line according to the World Bank. Majority of the inhabitants consume groundwater from dug wells and tube wells especially in West Bengal and its adjoining areas. The municipal water supply is polluted with arsenic rich iron oxyhydrides [1]. Chronic effects of arsenic include oxidative stress, mitochondrial dysfunction, liver damage and impaired glycemic control [2]. According to several survey reports, exposure to inorganic arsenic compounds may be associated with development of diabetes mellitus [3-7]. According to an Indian daily, India ranks among the top three countries in the world for the prevalence of diabetes. For an underdeveloped country like India, more than half of the population could afford the remedial drugs and depend generally on medicinal plants. Of which, a well-known medicinal plant, namely, *Costus igneus* or insulin plant has been reported to lower blood glucose levels on consumption of its leaves by diabetic patients [8,9]. It is called the magic cure for diabetes. Various researches reveal its different components like carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, steroid and appreciable amount of trace elements [10]. Moreover, it contains an orally active insulin like protein (ILP) having potent hypoglycemic activity [11].

The current study is an attempt to protect the rural population from arsenic contaminated groundwater induced hyperglycemia in a cost effective and natural way. In order to accomplish the motive in our preliminary investigation we have studied the following parameters: body weight, blood sugar level, histological study of pancreas and islet cell count on male albino rats.

Materials and Methods

Animals

The investigation was conducted after obtaining approval from

Institutional Animal Ethical Committee for Animal Experimentation (Ref. no. HMGC-/IAEC/M.R-2). Male albino rats aged 100-120 days of Wistar strains were obtained from, and maintained in, the animal house, Department of Physiology, Harimohan Ghose College, Kolkata, West Bengal, India. The animals weighed 120-140 g and had access to food and water *ad libitum*. They were kept at a temperature of 28 ± 4°C. The lighting schedule was maintained at 12 h of light per day, and the rats were acclimatized for 3 days before the beginning of the experiment.

Preparation of the plant product

The leaves of the insulin plant or *Costus igneus* were obtained from the campus of University of Calcutta, Department of Home Science in August, 2015 and authentication of the plant was done by the Department of Botany, University of Calcutta. The leaves were shade dried, grind in pestle and mortar and passed through sieve and stored in airtight container at refrigeration temperature (4°C) before use.

Treatment schedule

The study was carried out for the period of 30 days. The animals were divided into three equal groups with six animals in each group.

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Groups were designated as Group Control (C), normal mammalian saline fed; Group Treated 1 (T1), arsenic trioxide (Sigma Chemical Co., St. Louis, MD, USA) administered orally at the dose of 3 mg/kg body weight/day which is within the range of LD₅₀ of a 70 kg body weight human (1-4 mg/kg) and less than one-thirteenth of LD₅₀ value of rats (40 mg/kg) for 30 days [12-14]; and Group Treated 2 (T2), arsenic trioxide at the dose of 3 mg/kg body weight/day for 30 days along with powder of the leaves of *Costus igneus* at the dose of 500 mg/kg body weight/day [7] forcibly fed for the last 15 days of arsenic treatment.

Measurement of body weight

The body weight of the experimental animals of all the three groups was recorded on Day 1, Day 15 and Day 30 to analyze the effects.

Assessment of blood sugar level

The blood sugar levels (BSL) of Group C, Group T1 and Group T2 were recorded on Day 1, Day 15 and Day 30 by Glucose-Oxidase Peroxidase kit in protein free supernatant [15,16] provided by Sigma Aldrich Corporation, USA.

Histological study: After the treatment period was over, the animals were sacrificed following ethical procedures and protocols. The pancreas was collected and preserved in Bouin's solution for hematoxylin eosin staining. The tissues were observed and studied under microscope. The alterations in the morphology were taken under consideration. Furthermore, variations in the islets cell count were evaluated [13].

Statistical analysis

All values were expressed as mean \pm SEM. Data were analyzed by ANOVA (model 1 or fixed model) followed by Student's two tailed 't' test [17] to test for differences between control and treated experimental groups. Differences were considered significant when (*p<0.05).

Results

No significant difference in body weight was observed between initial, Day 15 and Day 30 of treatment in Group C rats, whereas the body weight of rats of Group T1 were decreased significantly on both Day 15 and Day 30 of treatment period compared to initial value (*p<0.05). On the other hand, the body weight of *Costus* supplemented rats (Group T2) was decreased significantly (*p<0.05) after 15 days but it also recovered to normal after 30 days in comparison to initial value (Figure 1). The BSL of Group C showed no significant difference on Day 15 and Day 30 from initial value. In Group T1, BSL were elevated significantly on Day 15 and Day 30 as well in comparison to initial value (*p<0.05). Conversely, Group T2 had significantly elevated BSL on day 15 (*p<0.05), but the level reduced almost to initial value on Day 30, as there was no significant difference in BSL between initial and Day 30 values (Figure 2). Histological study of pancreas demonstrated large number of islet cells in Group C and reduction in cell size in Group T1. The reduced cell morphology was restored to some extent in Group T2 (Figures 3a-3c). The islet cell count was decreased significantly in Group T1 compared with Group C (*p<0.05) whereas this value improved in Group T2 as there was no significant difference in cell count between Group C and Group T2 (Figure 4).

Discussion

The global problem of arsenic contamination in drinking water is imposing severe threat to human health [18-20] and several reports show development of diabetes mellitus is associated with arsenic

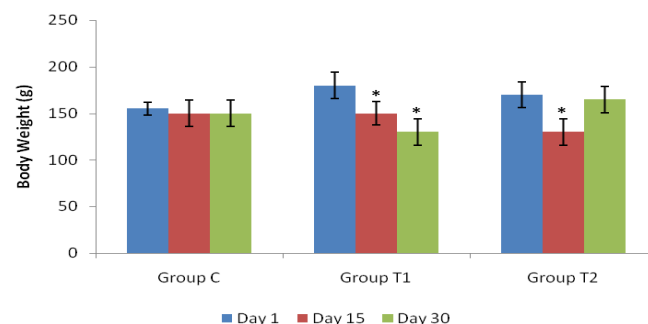


Figure 1: Effect of arsenic trioxide at the dose of 3mg/kg/day and simultaneous *Costus* leaves supplementation at the dose of 500mg/kg /day on body weight (g) of male rats at three different intervals.

[Values marked with asterisks are significantly different from corresponding initial values (values are mean + SEM of 6 rats/group)]

[ANOVA followed by Student's two tailed 't' test, where *p<0.05]

Group Control(C) normal mammalian saline treated

Group Treated1 (T1) arsenic trioxide fed at the dose of 3mg/kg/day for 30 days

Group Treated2 (T2) arsenic trioxide fed at the dose of 3mg/kg/day for 30days and *Costus* supplementation at the dose of 500 mg/kg/day for the last 15 days of arsenic treatment.

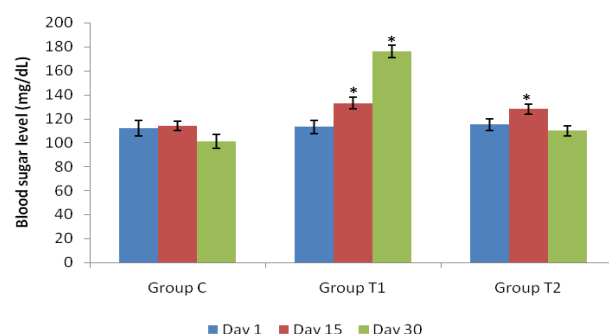


Figure 2: Effect of arsenic trioxide at the dose of 3mg/kg/day and simultaneous *Costus* leaves supplementation at the dose of 500mg/kg /day on blood sugar level (mg/dL) of male rats at three different intervals.

[Values marked with asterisks are significantly different from control values (values are mean + SEM of 6 rats/group)]

[ANOVA followed by Student's two tailed 't' test, where *p<0.05]

Group Control(C) normal mammalian saline treated

Group Treated1 (T1) arsenic trioxide fed at the dose of 3mg/kg/day for 30 days

Group Treated2 (T2) arsenic trioxide fed at the dose of 3mg/kg/day for 30days and simultaneous *Costus* leaves supplementation at the dose of 500 mg/kg/day for the last 15 days of arsenic treatment.

exposure [21-23]. Since, most of the arsenic affected populations of South Asia specially Bangladesh and West Bengal of India are belonging to economically weaker section of the society [1,20], expensive medicines for treatment of diabetes are unaffordable for them. As a result, alternative cheaper mode of treatment is in urgent need for these people.

In the present study, simultaneous supplementation of the leaves of *Costus igneus* to arsenic induced hyperglycemic rats resulted in

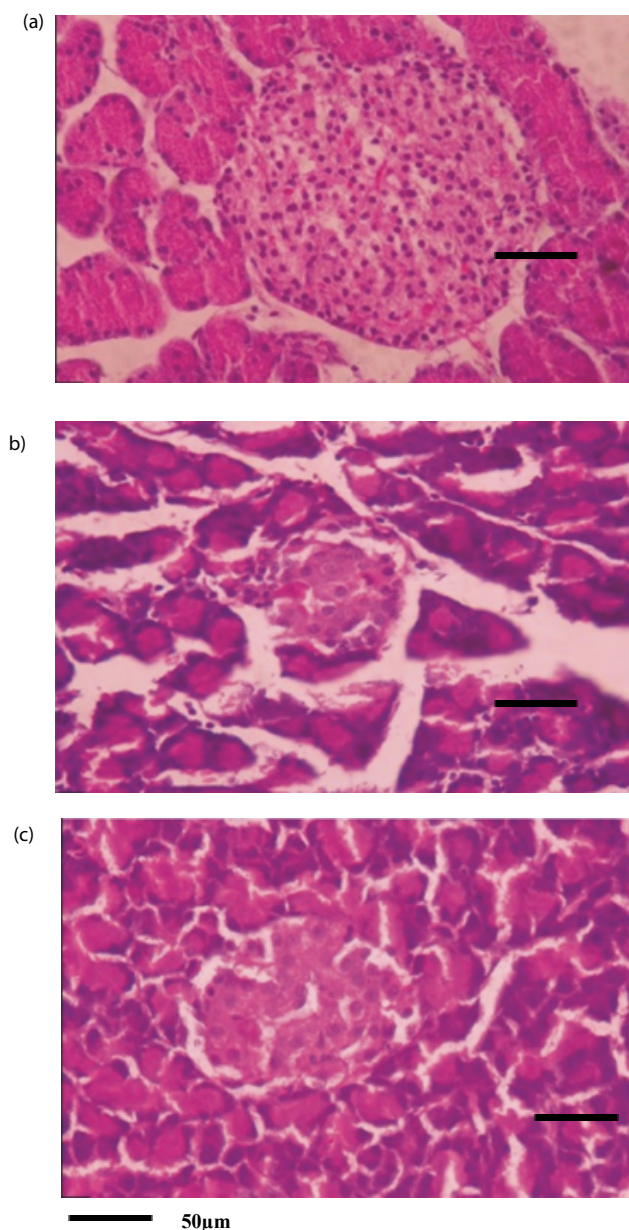


Figure 3: Photomicrograph of the pancreatic islets cell histology of the (a) control group rats (Group C); (b) rats treated with arsenic trioxide at the dose of 3 mg/kg/day for 30 days (Group T1); (c) rats treated with arsenic trioxide at the dose of 3 mg/kg /day for 30 days and simultaneous supplementation of leaves of *Costus igneus* at the dose 500 mg/kg/day from Day 15 to Day 30 (Group T2) of arsenic treatment.

improvement from disease conditions. Though there were very few scientific documentation regarding the hypoglycemic action of *Costus* [7,24], a section of hyperglycemic Indian population used to eat this leaf to keep their blood glucose low [6,25].

The body weights of arsenic treated group (T1) decreased significantly which is a general manifestation of diabetes mellitus occurring due to excessive breakdown of tissue proteins [26]. *Costus* supplemented rats retarded their weight loss (Figure 1) indicating an improved metabolic status from diabetes. The reduction of blood glucose concentration in simultaneous *Costus* supplemented group (T2)

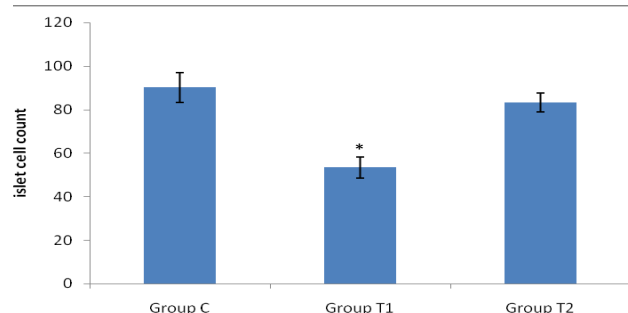


Figure 4: Effect of arsenic trioxide at the dose of 3mg/kg/day and simultaneous supplementation of leaves of *Costus igneus* at the dose 500mg/kg /day on islet cell count of pancreas among the groups of rats.

[Value marked with asterisk is significantly different from control value (values are mean + SEM of 6 rats/group)]

[ANOVA followed by Student's two tailed 't' test, where * $p < 0.05$]

Group Control(C) normal mammalian saline treatment

Group Treated1 (T1) arsenic trioxide fed at the dose of 3mg/kg/day for 30 days

Group Treated2 (T2) arsenic trioxide fed at the dose of 3mg/kg/day for 30days and simultaneous supplementation of leaves of *Costus* at the dose of 500 mg/ kg/day for the last 15 days of arsenic treatment.

from the hyperglycemic level of T1 signifies a protective effect of this plant from diabetes which is in agreement with the previous findings [7]. Moreover, arsenic induced hyperglycemia is possibly due to severe damage to the pancreas [27,28] evident by shrunken type morphology of islet cells in Group T1 [Photomicrograph 3(b)] in comparison to control pancreatic islet cells [Photomicrograph 3(a)]. *Costus* supplementation improved the cell morphology [Photomicrograph 3(c)]. The histological feature is also reflected in islet cell counts (Figure 4) which shows a significant increase in count in T2 compared to T1 indicating the hypoglycemic action of *Costus* to be mediated by improving pancreatic endocrine status.

The exact mechanism of action of this plant product on combating hyperglycemia cannot be determined from this preliminary investigation. Nevertheless, use of this plant as an alternative medicine by the arsenic exposed diabetic people may be encouraged as it is easily available, cheap and is proved to be beneficial. Future studies to elucidate the molecular mechanism of its action are urgently needed to establish the anti-diabetic potential of this plant material at least against arsenic induced hyperglycemia.

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Conflict of Interest

Authors have no conflict of interests to declare.

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