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Antidiabetic Evaluations of some Traditional Plants in Alloxan Induced Diabetic Mice Model

Md. Niamul Shahadat, Rakibul Islam, Mst. Misrat Masuma Parvez, Sumon Sarkar*

Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200.

**Corresponding Author: sarkarsumon.setu@gmail.com,*

Abstract: *Diabetes mellitus (DM) is defined as a group of metabolic diseases manifested by hyperglycemia which results from defects in insulin production and/or insulin action. The present study was conducted to demonstrate the diabetogenic effect of Alloxan in Mice. Synthetic drug treatment for diabetes mellitus carries risks that lead to many adverse effects such as weight loss, hypoglycemia and many others. Asian countries including Bangladesh, India are rich in natural resources and medicinal plants useful in the treatment of diabetes. To investigate the anti-diabetic or anti-hyperglycemic effect of the Bitter melon, Chirata and Cinnamon extract on Alloxan induced diabetes in experimental mice. The extract of bitter melon, chirata and cinnamon were tested for its efficacy in Alloxan at a dose 120mg/kg of body weight induced diabetic mice.This study was conducted to compare the anti-diabetic effect of Bitter melon, Chirata and Cinnamon extracts on body weight, blood glucose, HbA1c and insulin level in Alloxan induced diabetic mice.Eighteen male swiss albino mice were kept in six different groups and each group have three male swiss albino mice for 21 days. Group T_0 served as negative control; Group T1 Alloxan induced 120 mg/kg of body weight served as positive control; Group T2 were Alloxan induced 120mg/kg and mice treated with 5g/kg Metformin drug; Group T3 were Alloxan induced 120mg/kg and mice treated with 300mg/kg of Chirata extract; Group T4 were Alloxan induced 120mg/kg and mice treated with 300mg/kg Bitter melon extract and Group T5 were Alloxan induced 120mg/kg and mice treated with 300mg/kg Cinnamon extract. The effects of extracts on blood glucose, HbA1c and Insulin level were tested by bio-chemestry analyzer. Results were analyzed by using one-way ANOVA at a 1% level of significance.The blood glucose level was significantly (< 0.01) reduced at 300 mg/kg body weight of Bitter melon extract as compared to the diabetic group. The HbA1c level was greatly (< 0.01) reduced at 300 mg/kg body weight of Chirata extract as compared to the diabetic group. The insulin level was significantly (< 0.01) increased at 300 mg/kg body weight of Cinnamon extract as compared to the diabetic group.Reduction in the blood glucose level by Bitter melon extract, also reduction in the HbA1c level by Chirata extract and greatly increased in insulin level by Cinnamon extract indicates that these have anti-diabetic effect in Alloxan induced diabetic mice.*

Keywords: *Diabetes; Alloxan; Bitter melon; Chirata; Cinnamon*

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I. Introduction

Diabetes from the Greek word meaning a siphon as the body acts as a conduit for the excess fluid, and mellitus from the Greek and Latin for honey. Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1].Diabetes mellitus describes a group of metabolic disorders characterized by increased blood glucose concentration. People living with diabetes have a higher risk of morbidity and mortality than the general population. The global prevalence of diabetes in adults has been increasing over recent decades. In 1964, it was estimated that 30 million people had diabetes. Less than 40 years later, the WHO estimated that there were 171 million people living with diabetes. The International Diabetes Federation (IDF) estimated the global prevalence to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, and 382 million in 2013. The IDF Atlas methodology was substantially updated in 2011 to incorporate an analytic hierarchy process that formalized the methods to priorities the highest quality data from available sources. Each estimate was based on the latest data available. Conferring to the International Diabetes Federation Atlas guideline report, presently, there are 352million adults with impaired glucose tolerance who are at high threat of developing diabetes in the future. In 2017,it was expected that 425 million people (20–79 years of age)suffered from Diabetes mellitus (DM), and the number is expected to rise to 629 million by2045. There were 6.926.300 cases of diabetes in Bangladesh in 2017. A recent survey revealed that Some 80 lakh people in Bangladesh

suffer from diabetes. The dramatic increase in diabetes has occurred in all countries, and in rural as well as urban areas. Accurate global, regional, and country-level estimates and projections of diabetes prevalence are necessary for prevention and treatment strategies to be planned and monitored, and to assess progress towards reaching the targets set by the Global Action Plan for Non-Communicable Diseases and the Sustainable Development Goals followed by IDF, 2015.Diabetes affects approximately 26 million people in the United States, while another 79 million have prediabetes. An estimated 7 million people in the United States have diabetes [2]. A consequence of the disease is adverse effects on both the macrovascular and microvascular system. Diabetic complications associated with macrovascular diseases are atherosclerotic macrovascular disease and ischemic coronary heart disease. Diabetic complications related to microvascular disease include retinopathy, nephropathy, neuropathy, and peripheral vascular diseases [3]. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes based on American Diabetes Association [1]. The vast majority of diabetic patients are classified into Type 1 diabetes is caused by an absolute deficiency of insulin and Type 2 diabetes is caused by both impaired insulin secretion and resistance involving muscle, liver and adipocytes [4]. Over time, diabetic patients with imperfect management undergo micro and macro-vascular complexity including nephropathy, retinopathy, neuropathy and cardiovascular disease [5]. At present, only insulin and oral anti hyper- glycemic drugs are attainable for type 1 diabetes management [6]. Many oral hypoglycemic agents are available along with insulin for the treatment of diabetes, but these agents having side effects and some are ineffective in chronic diabetic patients [7]. There are some synthetic drugs which are mostly used for the treatment of diabetes ellitus and also the treatments are cost effective. In case of synthetic drugs there are also some side effects and long term use may cause increase the risk of cholesterol in bile duct, gallstone, insomnia, fat deposition and so on. In case of poor people, it is not easy to use synthetic drug for long term. According to a study by icddrb in 2016, diabetes patients had two times more days of inpatient treatment, 1.3 times more outpatient visits and 9.7 times more medications than those who don't have diabetes. The study found that annual per-capita expenditure on medical care was 6.1 times higher for diabetic patients than non-diabetic ones (USD 635 vs USD 104 respectively). Patients of type-1 diabetes, which is genetic, can't survive without insulin. “Having access to insulin, therefore, has become a human rights issue for those suffering from type-1 diabetes. People from low-income groups who cannot buy insulin have only one option: death, unless insulin is made available to them through charity.

From an economic perspective, the total annual cost of diabetes in 2012 was estimated to be 245 billion dollars in the United States. This included 116 billion in direct medical costs (healthcare costs) for people with diabetes and another 69 billion in other costs due to disability, premature death, or work loss. Medical expenses for people with diabetes are over two times higher than those for people who do not have diabetes. These numbers reflect only the population in the United States [8]. Medicinal plants are a major source of drugs for the treatment of various health disorders. Nowadays huge number of allopathic medicines also contains plant based ingredients that are used for their preparation by different companies. There are about 400,000 species of higher plants in the world, as compared to animal’s species that are about 5-10 million. The plant materials contain thousands of chemicals which act against diseases and infections of humans and animals when properly used [9]. A scientific investigation of traditional herbal remedies for diabetes may provide valuable treatment for the development of alternative drugs and therapeutic strategies. Alternatives are clearly needed because of the inability of current therapies to control all of the pathological aspects of diabetes and the high cost and poor availability of current therapies for many rural populations, particularly in developing countries [10]. Traditional medicine is still the most affordable and easily accessible source of treatment in the primary healthcare system. Medicinal plants have always been a potential source to cure different diseases, either in the form of traditional preparations or as pure active principles, and they are frequently the only source of medicine for the majority of people in the developing world. In this context, worldwide efforts have been taken to improve plant based therapies. WHO [11] recommended for the assessment of traditional medicinal plant in connection with the management of diabetes mellitus. Research on phytomolecules as diabetes remedies is upraising gradually as these are with minimal or no side effects [12]. Traditionally used medicinal plants are an essential part of the health sector in Bangladesh due to its abundance of a vast source of ethno-medicine. Rural people from developing country like Bangladesh are greatly dependent on traditional source of medicine. The prevalence of diabetes mellitus isincreasing from recent years; therefore, various researches are going on to discover better medicine and find out their traditional formulation as anti-diabetic medicine and their pharmacological activity has also been explored through literature search [13].

However, in Bangladesh the traditional medicinal plants that are used for the treatment of diabetes have not yet been studied in great details. Therefore, these herbal remedies are important objects of research,

especially in context of the virtually exploding prevalence of diabetes mellitus in Bangladesh. Not only very few research of comparative studies of medicinal plants on diabetes in the world but also Bangladesh. So the comparatively use of herbal medicinal plants such as bitter melon, cinnamon and chirata as the treatment for diabetes it is easy to use.In case of Bitter melon (*Momordicacharantia*), which belongs to family Cucurbitaceous, is an important vegetable mainly valued for its nutritional and medicinal properties. Bitter melon fruits also possess anti-oxidant, anti-microbial, anti-viral, anti-diabetic activities [14]. IT is widely available in Bangladesh and also well known as agent with several anti-diabetic effects. Numerous studies revealed that anti-hyperglycemic effects for its fruits in experimental animal studies of induced diabetes, but also the leaves, stem and seeds were used for anti-diabetic treatment [15]. The parts used include the whole plant, fruits and seeds which are bitter due to the presence of chemical momordicin [16]. The glucose lowering effect of its unripe fruit juice has been demonstrated in both experimental animal models [14] and human clinical trials[17]. Oral administration of bitter melon preparations also showed significant results when tried clinically in type 2 DM patients [17].

Cassia cinnamon (Cinnamoum cassia) is one of the popular spices of cinnamon, which is widely distributed in Asia especially in India, China, Bangladesh and Nepal [18]. Cinnamaldehyde and trans-cinnamic acid were active metabolites of cinnamon bark.).Some study was revealed that the active compounds of cinnamon (such as cinnamaldehyde, cinnamicacic, eugenol and other compounds)posseswide ranges of pharmacological effects that seems to be highly bioactive against diabetesbyits effect on insulin secretion and stimulate glucose uptake by hepatocytes and adipocytes [19]. Cinnamon has been shown to reduce insulin resistance, reduce blood glucose and lipid levels, reduce inflammation, and increase antioxidant activity [20]. *Swertia* species are common ingredients in a number of herbal remedies. This ethnomedicinal herb is known mostly for its bitter taste caused by the presence of different chemical constituents such as amarogentin (most bitter compound isolated till date), swerchirin, swertiamarin, and other bioactive compounds that are directly associated with human health welfare [21]. Based on research, scientists confirmed this plant as a potential antidiabetic agent. Recent decades researchers also revealed its antimalarial, anti-inflammatory, anti-oxidative, and anti-carcinogenic properties of this plant. *S. chirata*root extract (500 mg/kg) decreases glucose and insulin levels and also improves lipid levels which are almost similar to the effect produced by the standard drug metformin and pioglitazone. The treatment with indinavir produces elevated glucose, insulin, and lipid levels. The groups treated with *S. chirata*root extract showed improved glucose, insulin and lipid profile in Indinavir treated rats.

The present study was therefore carried out to evaluate the traditional used of bitter melon, cinnamon and chirata as anti-diabetic scientifically. Furthermore, the positive roles of natural products (neutraceuticals) for the correction and management of diabetes and other related complications, were also assessed.

II. Materials And Methods

2.1. Experimental Design

This experimental work was conducted during the period between 1 march to 30 march at animal laboratory under the department of physiology and pharmacology at Hajee Mohammad Danesh Science and Technology University, Dinajpur for a period of 30 days to evaluate the Comparative study of antidiabetic activity among three medicinal plants against alloxan induced diabetes in mice.

2.2 Experimental Animals and Study protocol

Laboratory male swiss albino mice (10-15) gm, 3 week of age, were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh is an international health research organization located in Dhaka, Bangladesh. All experimental animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by committee for purpose and control of supervision of experiment on Animals and approved by department of physiology and pharmacology. The animals were allowed to acclimatize in the laboratory environment for a week before the commencement of the experiment. The mice were housed in a wire cages measuring 30×13×15 cm at temperature $(25\pm2)^0$C and 12/12 light/dark cycle under controlled environment and sawdust substrate was changed weekly. The mice were fed a standard commercial pellet diet at a dose of (100-150) gm/kg recommended or advised by icddrb, in Dhaka and water adlibitum throughout the experimental period.

2.3 Design of Experiment

18 male swiss albino mice were used to carry out this investigation and the mice were divided into six groups comprising of six mice in each group designed and maintained as follows: Group T_0: The mice were fed normal diet and water supplied adlibitum and their body weight and blood glucose level were recorded after acclimatization. This group served as Negative control group. Body weights and glucose level were measured at the time when that of the other groups were measured. GroupT_1: After acclimatization, body weight and blood

glucose level were measured after 18 hours of starvation, then Alloxan induced by subcutaneous injection at a dose rate 120 mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied adlibitum water from day 1 to 21. After 48 hours' blood glucose level was determined by glucometer to ensure hyperglycemic or diabetic condition and the body weight were again measured. This group served as positive control group. Then all the mice of the group were kept for 21 days, during that period on day 1 and 21 the body weight and blood glucose level were measured. GroupT_2: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then Alloxan induced by subcutaneous injection at a dose rate 120 mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied adlibitum water from day 1 to 21. Then the blood glucose level and body weight were measured of alloxan induced to mice by subcutaneous injection to ensure diabetic condition. After that standard anti diabetic drug of motorman (trade name=comet) was given for 21 days. During treatment of metformin drug body weight and blood glucose level were recorded on 1 and 21 days. This group served as treatment group to find the effect of treatment with 5 mg/kg body weight of metformin. Group T_3: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then Alloxan induced by subcutaneous injection at a dose rate 120 mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied adlibitum water from day 1 to 21. Then the blood glucose level and body weight were measured of alloxan induced to mice by subcutaneous injection to ensure diabetic condition. After that extraction of Swertiachirata was given for 21 days. During treatment of swertiachirata extract body weight and blood glucose level was recorded on 1 and 21 days. This group served as treatment group to find the effect of treatment with 300 mg/kg body weight of swertiachirata extract. Group T_4: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then Alloxan induced by subcutaneous injection at a dose rate 120 mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied adlibitum water from day 1 to 21.Then the blood glucose level and body weight were measured of alloxan induced to mice by subcutaneous injection to ensure diabetic condition. After that extraction of bitter melon was given for 21 days. During treatment of bitter melon extract body weight and blood glucose level were recorded on 1 and 21 days. This group served as treatment group to find the effect of treatment with 300 mg/kg body weight of bitter melon extract. Group T_5: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then Alloxan induced by subcutaneous injection at a dose rate 120 mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied adlibitum water from day 1 to 21. Then the blood glucose level and body weight were measured of alloxan induced to mice by subcutaneous injection to ensure diabetic condition. After that extraction of Cinnamon was given for 21 days. During treatment of Cinnamon extract body weight and blood glucose level were recorded on 1 and 21 days. This group served as treatment group to find the effect of treatment with 300 mg/kg body weight of Cinnamon bark extract. Concentration selections were based on the safe doses of extract in oral acute toxicity studies carried out earlier in this study Chirata, Bitter melon, Cinnamon extracts and Metformin were administered orally for 21 days. Instruments, Reagents, Drug and Chemicals. The following instruments, reagents and drugs were used for this study.

2.4 Induction of Experimental Diabetes

Diabetes were induced by giving subcutaneous freshly prepared alloxan powder 120mg/kg dissolved in 0.5 ml acetate buffer (ph5.5) in a volume of 24ml/kg body weight to an overnight fasting of the animals. After 48 hours of Alloxan induction, fasting blood glucose level was determined by glucometer and mice with blood glucose levels 200mg/dl were considered as diabetic and used for the experiments. Alloxan can selectively destroy the pancreatic B-cells with rapid and irreversible necrosis and can be used to generate a chronic model of hyperglycemia with diabetic complications.

Protocol:

- Administer an subcutaneous injection of Alloxan (120mg/kg body weight).
- Monitoring the glucose level for onset of hyperglycemia.

Collection, Preparation, Preservation and Administration of Chirata, Bitter melon and Cinnamon Extract

Collection:

Chirata root, Bitter melon fruit and Cinnamon bark were purchased from the local market in Dinajpur at a reasonable price.

2.5 Preparation and Preservation of extract:

2.5.1 Chirata extract

The sample preparation was followed by Rajesh *et al*., (2017) [22] with some modification. Chirata root was collected from the local market in Dinajiur. The roots were washed carefully with distilled water to remove any extraneous material. The roots were shade dried at room temperature and the grounded to course

powder using electric grinder. Then the ethanol is used for the process of extraction and stored in refrigerator until use.

2.5.2 Bitter melon extract

The sample preparation was followed by [23] with some change. The cleaning of Bitter Melon was made using distilled water before cutting them into small pieces and then oven dried at 50°C for a day. The dried sample was then pulverized into fine powder in an electric grinder extracted (by maceration method in 70% ethanol) and concentrated (using rotary evaporator) under reduced pressure which was then stored at 4°C in refrigerator until use [23].

2.5.3 Cinnamon extract

The procedure was followed by [24] with some modification. Cinnamon bark was collected from the local market in Dinajpur. The bark was washed carefully with distilled water to remove any extraneous material. The bark was oven dried at 50°C for a day and grounded to course powder using electric grinder. Then five hundred grams of grinded powder cinnamon was soaked in 1000 ml ethanol for 7 days with occasional shaking, and then filtered. The filtrate was condensed by rotatory vacuum evaporator and stored in refrigerator.

Administration of Chirata, Bitter melon and Cinnamon extract

Procedure

Prepared extract of chirata roots, bitter melon fruits and cinnamon bark were fed orally to different treatment groups to the experimental mice with the help of a micropipette. The use of micropipette ensured the administration of requisite quantity, which was ascertained on the basis of body weight of each individual mice.

2.6 Toxicity evaluation in mice

The extracts were tested for the acute toxicity (if any) in mice. To determine the acute toxicity of a single oral administration, different doses of the formulation (0.5, 1.0,1.5 and 2 g/kg BW) were administered to different groups of the mice (three mice were used for each group). Mortality and general behavior of the animals were observed continuously for the initial four hrs and intermittently for the next six hrs. and then again at 24 hrs and 48 hrs following dose administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions.

2.7 Recording of Different Parameters

2.7.1 Determination of body weight

Body weight was taken on day 1 and 21st day of treatment (During treatment)

Procedure:

Body weight of all groups were recorded before treatment on 1st day and the effect of the treatment at the end of the experiment on 21st day by the help of electric balance.

2.8 Recording of Blood Glucose Level

2.8.1 Collection of Blood

Procedure:

For time to time blood glucose level determined, the blood samples were collected from the tail vein of each mice of group as a drop. The drop was then immediately placed on the strip of the Glucolab ® active monitor to find the glucose level quickly. The values were expressed in mmol/L.

2.9 Determination of Blood Glucose Level:

Blood samples were collected from the tail vein at day 0 (Pre-treatment) and 21st day at the end of study period for estimation of blood glucose level. Estimation of blood glucose level was performed by Glucolab® active monitor blood glucose system (Strip method).

2.10 Determination of HbA1c level

At the end of the study period, all groups of mice were euthanized by anesthetizing with diethyl ether and then blood sample was collected in vial without anticoagulant via lateral tail vein. After the blood sample was immediately transferred to the popular diagnostic center limited in Dinajpur, Bangladesh for the estimation of HbA1c level. Estimations were carried out by BIO-RAD, D-10 (USA) HPLC analyzer. Then the Reports were delivered for further analysis.

2.11 Determination of Insulin level

At the end of the experimental period, all groups of mice were euthanized by anesthetizing with diethyl ether and then blood sample was collected in vial with anticoagulant via lateral tail vein. After the blood was centrifuged for 10 minutes at 3000 rpm by the centrifuge machine. Then the serum samples were immediately

transferred to the popular diagnostic center limited in Dinajpur, Bangladesh for the estimations of insulin level. Estimations were carried out by Architect l-1000SR/VitrosECi System (J andJ) Random Access Multibatch Immunoassay Analyzer. Then the Reports were delivered for further analysis.

2.12 Statistical Analysis

The results of various biochemical and immunological parametrs were expressed as ±SEM. Data analysis of the Statistics were done using SPSS version 22 and Microsoft Excel. Statistically significant differences between group means were determined by analysis of variance (ANOVA).

III. Results

The table 1 showed that the initial body weight Body weights of different groups were almost same. But the final body weights of these groups were varied significantly. The present study indicated that the final body weight of positive control (diabetes induction) T_1 (26.33±0.88) was significantly decreased from the treatment of negative control T_0 (36.00±1.15), metforming T_2 (37.08±1.15), chirata T_3 (36.33±0.88), bitter melon T_4 (40.00±1.15) and cinnamon T_5 (36.67±1.20). In the table 2 the result showed that the blood glucose level**,** HbA1c level and insulin level. The present study revealed that the blood glucose level was significantly decreased in the negative control (no diabetes) T_0 (4.13±0.35), metformin treatment T_2 (4.03±0.38) and bitter melon treatment T_4 (5.63±0.81b) from the positive control (diabetes) T_1 (8.50c±0.66). However, no significant differed were observed at the treatments of chirata T_3 (8.17±0.60c) and cinnamon T_5 (8.40±0.79) from the positive control. In this study result also indicated that the Hba1c levels were significantly decreased at the treatments of cinnamon T_5 (4.50±0.29), metformin T_2 (4.63±0.33) and negative control T_0 (4.87±0.52)from the positive control (diabetes) T_1 (6.17±0.12). However, no significant different were found at the treatments of bitter melon T4 (5.73±0.15) and chirata T_3 (6.0±0.12) from the positive control. This study also observed that the insulin levels were significantly decreased at the treatments of positive control T_1 (8.00±0.58), cinnamon T_5 (8.17±0.73), bitter melon T_4 (8.50±0.15) from the negative control T_0 (15.00±0.58) and no significant different were observed at the treatments of chirata T_3 (11.33±0.33) and metformin T_2 (12.33±0.33) from the negative control.

IV. Results And Discussion

The experiment was conducted to determine the comparative efficacy of anti-diabetic activity of synthetic (Metformin) and herbal (Chirata, Bitter melon and Cinnamons) drugs on body weight, blood glucose, HbA1c and insulin levels in alloxan induced diabetic mice. It was also compared the different herbal drugs on body weights, blood glucose, HbA1c and insulin levels in alloxan induced diabetic mice. To perform the experiment, 18 mice were randomly divided into six equal groups named T_0,T_1, T_2,T_3, T_4 &T_5 and each group containing 3 mice. Alloxan was injected subcutaneously at the dose rate of 120mg/kg body weight to the groups (T_1, T_2,T_3,T_4,T_5) mice for induction of diabetic syndrome. Group T_0 mice were kept as non- diabetic (negative) control without giving alloxan and any other treatment. Group T_1 mice were kept as diabetic (positive) control without giving any therapeutic agent. Group T_2 mice were treated with synthetic drug metformin (5mg/kg) body weight. Groups of miceT_3dose rate of 300 mg/kg for consecutive 21 days, respectively after confirming diabetic condition,T_4 &T_5were treated with aqeous extract of chirata, bitter melon and cinnamon at the. All groups were closely observed during 21 days of treatment period.

4.1 Body weight

The present study indicated that the final body weight of positive control (diabetes induction) T_1 (26.33±0.88) was significantly decreased from the treatment of negative control T_0 (36.00±1.15) and similarly followed by metforming T_2 (37.08±1.15), chirata T_3 (36.33±0.88), bitter melon T_4 (40.00±1.15) and cinnamon T_5 (36.67±1.20). In the present study, it was found that the final body weight of negative control(no diabetes) and different antidiabetic treatment groups were significantly increased ($P<0.01$) from the positive control groups(diabetes) that showed similarity with the findings of [25]. He found that the body weight was significantly increased in the bitter melon treated diabetic mice. The results were also supported [26] who reported that the body weight was lowered by 5 to 25% in diabetic control group but was higher in extract of momordicacharantia treated rats by 6.60 to 30%.The present findings were in the disagreement with the findings of Farhatbano *et al.,* (2011) [27] reported that aqueous extract of momordicacharantia significantly reduced body weight(303±7.03 vs 253.72±7.8gm). The present study revealed that the body weights were insignificantly increased in the treatments of cinnamon, chirata and Metformin of diabetic mice. This study was similarity with the findings of Gaber*et al.,* (2012) [28] who reported that the cinnamon administered showed significantly increase in body weight of diabetic rats.These findings which are dissimilar with Auddy *et al*, (2003) [29]. The protective effect of cinnamon extract on body weight loss may be due to its ability to reduce hyperglycemia.Here the bioactive compounds of cinnamon may help in suppressing the free radicals generated

and control over muscle wasting resulted from glycemic control in treated diabetic mice and ultimately lead to normalize the level of body weight. This study also showed similarity with the findings of [30] who reported that daily treatment with the plant of ethanol extract of swertiachirata and andrographispaniculata supplement at 200 mg/kg body weight to diabetic groups.The animal resulted significantly increases in body weight at the end of the treatment compared to diabetic groups. The study revealed that the decreased amount of the final body weight by alloxan injected subcutaneously at the dose rate of 120mg/kg body weight of mice for induction of diabetic syndrome. The present results were in the agreement with [28] who reported that decrease in body weight by alloxan induced in diabetic rats was possible due to catabolism of protein and fats even though the food intake was more in diabetic rats than control.

4.2 Glucose level

The present study revealed that the blood glucose level was significantly decreased in the negative control (no diabetes) T_0 (4.13±0.35), metformin treatment T_2 (4.03±0.38) and bitter melon treatment T_4 (5.63±0.81b) from the positive control (diabetes) T_1 (8.50c±0.66), chirata T_3 (8.17±0.60c) and cinnamon T_5 (8.40±0.79). The present study indicated that the blood glucose levels were significantly increased in the treatment of positive control group (T_1), chirata(T_3)and cinnamon group (T_5) which were compared to the negative control group (T_0). This result showed that the blood glucose levels were significantly ($P<0.01$) decreased in the treatments of bitter melon and metformin groups compared to the other treatment groups.These observations similar to the findings of Tariqul Islam *et al.,* (2012) [31] who reported that administration of glibenclamide (5 mg/kg)significantly reduced blood glucose level in alloxan induced hyperglycemic mice. Similar observations were made by Begum*et al.,* (2012) [32] who used the same dose of glibenclamide in alloxaninduced diabetic mice and found the reduction of bloodglucose level from 9.99 mmol/L to 4.99 mmol/L.These result supported to the findings of Miura *et al.,* (2001) [33] who reported that significantly reduction of blood glucose level ($P<0.01$) in diabetic mice treated by momordicacharantia. this finding are also in agreement with [34] who reported that ethanolic extract of dried momordicacharantia in alloxan induced albino rats showed significantly ($P<0.05$) lowering the blood glucose level. This study observed that whole momordicacharantia significantly reduce blood glucose level. These observations are in disagreement with [35] who reported that extract of momordicacharantia without seeds caused 20% fall in blood sugar level of diabetic rabbits.In this study it was revealed that the slightly decreases in blood glucose level was found in T_3 which are treated with chirataextract as like as T_1 (diabetic) group. The present results which are similar to the [36] who reported that the ether extraction of swertiachirata also shows significant hypoglycemic activity on swiss albino mice in relative to control and standard groups.It was also observed that the slightly decreases in blood glucose level was found in T_5 which are treated with cinnamon extract as like as T_1 (diabetic) group. The present findings were the related of SteaveBelvin *et al.,* (2007) [37, 38] who reported that using cinnamon had no significant effect on glucose level in type 2 diabetes. The present investigations were not related to the results of Ping *et al.,* (2010) [39] who founded that fasting blood glucose concentration was significantly decreased ($P<0.05$) compared with the diabetic control group and Soni*et al.,* (2009) [40] who reported that using cinnamon had significant effect on the lowering blood glucose of type 2 diabetes.

4.3 HbA1c level

In this study result also indicated that the Hba1c levels were significantly decreased at the treatments of cinnamon T_5 (4.50±0.29), metformin T_2 (4.63±0.33) and negative control T_0 (4.87±0.52) from the positive control (diabetes) T_1 (6.17±0.12) and other treatments of bitter melon T4 (5.73±0.15) and chirata T_3 (6.0±0.12). The findings of the present study showed that the lower HbA1c level was significantly found in T_5 and T_2 which are treated with cinnamon extract and metformin as like as normal group compared to non-treated diabetic group. These results are the similar of Suppapitiporn (2006) [41] who reported that administration of cinnamon extract or metformin showed that decreases in HbA1c was more than double in cinnamon group but decreases were not significant. Pauline *et al.,*(2016) [42] who recommended that cinnamon can be effective therapy for reducing HbA1c level in type 2 diabetes. Also similar to the [43] who reported that cinnamon lowers HbA1c in type 2 diabetes. The present results are not related to the result of Dugoua*et al.,* (2007) [44] who reported that the value of glycosylated hemoglobin reduced in cinnamon treated groups respectively, this reduction was non-significant compared to base line value.In this study, it was revealed that the insulin level was slightly decreased in T_4 and T_3 varied to non-treated T_1group.This results which are in line to the results with [45] who reported that the addition of bitter melon capsule could decrease HbA1c level by 1% compared to the non-treated groups.

4.4 Insulin level

This study also observed that the insulin levels were significantly decreased at the treatments of positive control T_1 (8.00±0.58), cinnamon T_5 (8.17±0.73), bitter melon T_4 (8.50±0.15) from the negative control T_0 (15.00±0.58) and other treatments of chirata T_3 (11.33±0.33) and metformin T_2 (12.33±0.33).This study was

indicated that highest insulin level in T_2 and T_3 as like as T_0 varied to another groups. This observation is agreed with Heather *et al.,* (2014)[46] who reported that aqueous extract of chirata stimulated insulin secretion in a concentration dependent manner from BRIN BD11 cells without affecting cell viability. [22] who showed that there was no significant deference on insulin and lipid profile between the groups treated with metformin and the combination of metformin with chirata root extract. The present results disagreement with [22] who reported that the group treated with swertiachirata root extract decrease insulin level and also improved lipid level which are almost similar to the effect produced by the standard drug metformin and piogliatazone.The present findings are in the agreement with the findings of Sarkar *et al.,* (1996) who reported that alcoholic extract of unripe momordicacharantia fruit significantly decrease the plasma glucose level ($P<0.01$) but insulin secretion not changed in diabetic rats. And Miura *et al*., (2001) [33] who showed that momordicacharantia significantly ($P<0.01$) lowering of serum insulin level in diabetic mice.In this study, it was observed that the T_5 groups which are treated with cinnamon extrat also showed insulin activity varied to the non-treated diabetic group.This results are similar with the results of [20] who observed that the aqueous extract to cinnamon containing polyphenol purified by high performance liquid chromatography showed insulin like activity.

V. Tables

Table1: Effects of synthetic (Metformin) and herbal (Chirata, Bitter melon and Cinnamon) drugs on Body weight in mice

Experimental groups of mice	Body weight(g)	
	Initial body weight	Final body weight
T_0	18.0±2.08	36.00^{b}±1.15
T_1	17.67±1.45	26.33^{a}±0.88
T_2	20.00±0.58	37.08^{c}±1.15
T_3	18.00±1.54	36.33^{b}±0.88
T_4	18.00±1.54	40.00^{c}±1.15
T_5	20.67±0.88	36.67^{b}±1.20
P value	0.484	0.00

Legends: Mean in each column with different superscripts were significantly different at $p<0.01$.
N.B: T_0=Negative control (No diabetes). T_1=Positive control (Diabetes induced). T_2=Diabetes with Metformin (5gm/kg). T_3=Diabetes with Chirata (300mg/kg). T_4=Diabetes with Bitter melon (300mg/kg). T_5=Diabetes with Cinnamon (300mg/kg).

Table 2: Effects of synthetic (Metformin) and herbal (Chirata, Bitter melon and Cinnamon) Drugs on Blood Glucose, HbA1c and Insulin level in mice

Parameter	Group						
	T_0	T_1	T_2	T_3	T_4	T_5	Pvalue
Glucose level (mmol/L)	4.13^{a}±o.35	8.50^{c}±o.66	4.03^{a}±0.38	8.17^{c}±0.60	5.63^{b}±0.81	8.40^{c}±0.79	0
HbA1c level (%)	4.87^{ab}±0.52	6.17^{c}±0.12	4.63^{a}±0.33	6.0^{c}±0.12	5.73^{bc}±0.15	4.50^{a}±0.29	0.004
Insulin level (µIU/ml)	15.00^{c}±0.58	8.00^{a}±0.58	12.33^{b}±0.33	11.33^{b}±0.33	8.50^{a}±0.29	8.17^{a}±0.73	0.00

Legends: Mean in each column with different superscripts were significantly different at $p<0.01$.
N.B: T_0=Negative control (No diabetes). T_1=Positive control (Diabetes induced). T_2=Diabetes with Metformin (5gm/kg). T_3=Diabetes with chirata (300mg/kg). T_4=Diabetes with Bitter melon (300mg/kg). T_5=Diabetes with Cinnamon (300mg/kg).
NS = Non significance

VI. Conclusion

In this study, final body weights of different treatments have showed significantly increased from the initial body weight. Bitter melon showed significant hypoglycemic effect in alloxan induced mice. Chirata also showed significant reduced HbA1c level in alloxan induced mice. On the other hand, Cinnamon showed also better results by increasing insulin level in alloxan induced mice. Generally, from the above findings it is possible to conclude that extract of bitter melon, chirata and cinnamon have anti-hyperglycemic and 300mg/kg extract of bitter melon, chirata and cinnamon have a better antidiabetic effect and almost equipotent with Metformin drug.

We could consider Bitter melon, Chirata and Cinnamon for diabetic patients as a practical choice for reducing blood glucose, HbA1c and insulin level. It can be recommended that further study can be done to investigate the combined anti-diabetic effect of bitter melon, chirata and cinnamon on lipid profile and also see the histo-pathological effect on pancreas, liver, kidney to make the study more comprehensive.

Conflict of interest
None to declare.

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