Effect of aqueous extract of chirata in alloxan-induced diabetic rat model

Protik kumar Sarker 1, Rakibul Islam 1, Bazlar Rashid 1, Sumon Sarkar 1, Misrat Masuma Parvez 1, Niamul Shahadat 1, Fahima Binte Aziz 1, Mushfika tabassum 3 and Saroj Kumar Yadav 2, *

1 Department of Physiology and Pharmacology, Faculty of Veterinary and Animal science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.
2 Department of medicine and surgery, Chittagong veterinary and animal's sciences university, Chittagong, Bangladesh.
3 Faculty of veterinary medicine, Chittagong veterinary and animal's sciences university, Chittagong, Bangladesh.

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Abstract

The present study was performed for evaluating the anti-diabetogenic effect of chirata extract in alloxen induced rat model which has rarely been done by any researcher previously. Diabetes was induced by injecting Alloxen (ALX) @ 120 mg/kg body weight subcutaneously to swiss albino mice. The sixteen rats were divided into four groups named Group A (non-diabetic) for the negative control, Group B (diabetic) for positive control, Group C treated with aqueous extract of chirata@ 125 mg/kg body wt and Group D treated with metformin (anti-diabetic drugs) @150 mg/kg body wt.). The duration of the experiment was 16 (sixteen) days. Finally, the research showed that the final body weight of positive control (diabetes induction) Group B (43.0±0.90) was significantly decreased from the treatment of negative control Group A (43.5±1.80) and similarly followed by treatment with a synthetic anti-diabetic agent such as metformin group D (44.50±1.70) and herbal drugs as chirata treatment Group C (44.0±1.95). The creatinine level in Group A, B, C and D were 0.85±0.07 mg/dL, 1.15±0.07 mg/dL, 0.95±0.08 mg/dL and 0.9±0.14 mg/dL respectively. The SGPT level in Group A, B, C, and D was 19±1.41 U/L, 27.44±2.82 U/L, 26.5±2.12 U/L, and 20.5± 2.12 U/L respectively. The SGOT level in Group A,B,C and D were 30±2.82 g/dL, 70±1.41 g/dL, 20.5± 2.12 U/L and 45±2.83 g/dL respectively. In alloxen-induced mice, a fall in SGPT levels was observed in the groups treated with aqueous extract of Chirata and Metformin. Aqueous extract of Chirata decreased SGOT levels compared with a diabetic control group. The body weight of the swiss albino mice was increased significantly in those treated with chirata extraction in the diabetic group. The synthetic drug metformin-treated group improved the body weight of the swiss albino mice model. The antihyperglycemic activity of aqueous extract of Chirata is comparatively lower than the synthetic anti-diabetic drugs such as metformin. Thus the above observations suggested that extracts, from Swertia chirata possess anti-diabetic principal and can presumably be used for the treatment of diabetes mellitus.

Keywords: Chirata; Anti-diabetogenic; Rat model; Traditional plants

1. Introduction

Diabetes mellitus is a major public health problem. The prevalence of diabetes is more pronounced in third-world countries like Bangladesh. In 2000, about 3.2 million people in Bangladesh were recognized to have diabetes and this ranked 10th position in global consideration. It is estimated that in 2030 about 11.1 million people in Bangladesh will be affected by diabetes which will rank the 7th position in global consideration [21]. There are many anti-diabetic drugs on the market and they performed good results as anti-diabetic drugs but to protect from side effects people are increasing their interest in herbal for disease effectively [1]. However, a large number of medicinal plants still remain to be investigated for their possible pharmacological values. The chirata is a herbal plant [14]. which have medicinal

*Corresponding author: Saroj Kumar Yadav

Department of medicine and surgery, Chittagong veterinary and animal's sciences university, Chittagong, Bangladesh.

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properties, particularly blood glucose lowering activity. Chirayata, are some of the most effective and the most commonly studied Indian plants in relation to diabetes. and many others have been shown to have anti-diabetic activity [3,9]. The plant extract chirata have been reported to possess anti-inflammatory [6], antipyretic [5], anti-viral [19], anthelmintic [7], antineoplastic [10]. In this study, an aqueous extract of Swertia chirata leaf was investigated to find out hypoglycemic activity on alloxan-induced Swiss albino mice with the following specific objectives.

- To evaluate the effect of chirata extract on body weight in diabetic rat.
- To determine the effect of chirata extract on blood glucose level, serum creatinine, SGPT and SGOT in diabetic rat.

2. Material and methods

2.1. Experimental Site

This research work was conducted at research shed under the department of Physiology and Pharmacology at Hajee Mohammad Danesh Science and Technology University, Dinajpur for a period of 16 days to evaluate the efficacy of chirata extract on alloxen-induced diabetes in rats.

2.2. Experimental Layout

2.3. Management of Mice

Fifteen (15) days of mice were collected from the ICDDRB, Mohakhali, Dhaka. Sixteen (16) mice were selected for the conduction of the research. When the research was started then the age of the mice became thirty (30) days. Thirty (30) days of sixteen (16) mice are divided into four (4) groups. Each group contains four (4) mice and weighing between 14-16 gm.

2.4. Acclimatization of Mice

All the mice were housed in screen-bottomed wire cages arranged in rows and kept in the departmental (Physiology and Pharmacology, HSTU) animal house. The animal was fed with pellet at a recommended dose of 100gm/kg body weight as advised by ICDDRB. Drinking water was supplied ad-libitum. The mice were maintained in this condition for a period of four weeks to acclimatize them prior to experimental uses.
Figure 2 Acclimatization of Mice in wire cages in the lab of the HSTU

2.5. Experimental Mice Grouping

Sixteen (16) mice were used to carry out this investigation. These mice were divided into four groups containing four (4) in each group. The groups were designated and maintained as follows by feeding as followings:

2.5.1. Group-A

The mice were fed a normal diet and given water ad-libitum and then their body weight and blood glucose was recorded after acclimatization. This group of mice served as the normal mice (Negative Control) group. Body weights and blood glucose levels were measured at the time when that of other groups was measured. This group served as a negative control group.

2.5.2. Group-B

After acclimatization, body weights and blood glucose levels were measured after 18 hours of starvation. Then Alloxen hydrochloride injection was given at a dose of 120 mg/kg bd. wt in intramuscular route to each mouse to induce diabetes.

The mice were fed a normal diet and given water ad-libitum from day 30-46 days without anti-diabetic treatment. This group served as the diabetic control group.

2.5.3. Group-C

After acclimatization, body weights and blood glucose level were measured after 18 of starvation. Then Alloxen hydrochloride injection was given at a dose of 120 mg/kg bd. wt in intramuscular route to each mouse to induce diabetes.

The mice were fed a normal diet and given water ad-libitum from day 30-46 days with chirata extract treatment at the rate of 125mg/kg bd wt.

Then on 15th-day blood samples were collected and measured for blood glucose levels, SGPT, SGOT, and creatinine levels from Islami Medical College Hospital, Dinajpur.

2.5.4. Group-D

After acclimatization, body weights and blood glucose levels were measured after 18 hours of starvation. Then Alloxen hydrochloride injection was given at a dose of 120 mg/kg bd. wt in intramuscular route to each mouse to induce diabetes.
The mice were fed a normal diet and given water ad-libitum from day 30-46 days with metformin treatment at the rate of 150mg/kg bd wt. (Intramuscularly)

Then on 16th-day blood samples were collected and the measurement of blood glucose levels, SGPT, SGOT, and creatinin levels were from Islami Medical College Hospital.

2.6. Preparation and Administration of Alloxen Solution
The alloxen (Alloxan monohydrate, sigma, Aldrich chemical) solution was prepared after weighing with the help of electric balance in the following ways: Alloxen (120 gm) was measured with the help of electric balance and dissolved into the 5 ml of acetate buffer solution and it was mixed well. Then 0.2 ml solution was injected intramuscular route into the body of the mice. Before giving Alloxen, the normal blood glucose levels of all mice were estimated. After 18 hours of treatment, if a diabetic condition could not be found then this solution was injected intramuscularly again to mice and maintained fasting condition for 18 hours. To induce diabetic condition in mice in, a dose of 120 mg Alloxen per kg body weight was chosen.

2.7. Preparation of chirata extract
Plant material and collection of chirata: Fresh leaves of Swerchia chirata (Chirata) were collected from the local market.

Extraction procedure of Swerchia chirata

2.7.1. Preparation of chirata extract
Extraction is the separation of medicinally active portions of plant and animal tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form and are intended for oral or external use.

Extraction is the first pre-purification step in the isolation and characterization of active compounds of a medicinal plant.

2.8. Collection
The leaf of Swerchia chirata will be collected from the Kalitola market at Dinajpur Sadar, Dinajpur, Bangladesh. The plant will be collected in February 2019 during the daytime. During collection, any type of adulteration will be strictly prohibited.

The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that a solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal.

2.9. Methods for Preparation of Chirata Extract

2.9.1. Extraction at a Glance
The aqueous extract was prepared according to method described by [11], with slight modification. The *Swertia chirata* leaves were dried and grinded into powder. The aqueous extract *swertia chirata* was performed due to there is very less research with aqueous extract of Chirta.

Each 100 g portions of powdered *Swertia chirata* sample was soaked with 200 mL distilled water and kept for two days with occasional stirring and then filtered. Three portions of filtrates were mixed together and evaporated to slurry in a rotary vacuum evaporator at temperature around 45°C. The slurry mass was then freeze-dried and stored in refrigerator till its application.

### 2.10. Preservation

All prepared Chirata extract was preserved in refrigerator at 0-4 degree celcious at Physiology and Pharmacology laboratory.

### 2.11. Determination of Parameters

After 16 days of treatment the mice were sacrificed for blood serum collection for further analysis

- Measurement of body weight
- Estimation of blood glucose level
- Estimation of blood creatinin level
- Estimation of blood SGPT
- Estimation of blood SGOT

#### 2.11.1. Determination of Body weight

Body weight was taken on day 30 days and 46 days.

#### 2.11.2. Estimation of Blood Glucose

![Figure 3 Determination of blood Glucose level with the help of Glucometer](image)

#### 2.11.3. Collection of blood

Procedure

For time to time blood glucose level determination, the blood samples were collected from the tip of the tail of the mice as a drop. The drop was then immediately placed on the strip of the Glucolab(R) active monitor to find the glucose level quickly.

#### 2.11.4. Determination of Blood Glucose Level

Blood samples were collected from the tip of the tail of the mice at 0 (Pre-treatment) days, 7 days, 14 days for estimation of blood glucose level was performed by Glucolab (R) Active monitor blood glucose system (strip method).
3. Results

Effect of Chirata extracts on body weight, creatinine level, SGPT, and SGOT in Rat at the end of the experiment.

The body weight of different groups of experimental mice was almost the same initially. But finally, the body weight of different experimental mice varied significantly. The initial body weight of experimental mice of Group A, B, C, and D for 42.9±1.76, 43.25±1.06, 43.75±1.80, and 44.0±1.65 respectively. The final body weight of an experimental group of mice was A, B, C, and D at 43.5±1.80, 43.0±0.90, 44.0±1.95, and 44.50±1.70 respectively.

Table 1 Effects of synthetic (Metformin) and herbal (Swertia Chirata) drugs on body weight on 30th and 46th days of age

<table>
<thead>
<tr>
<th>Experimental group of mice</th>
<th>Body weight (gm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Body weight</td>
<td>Final Body weight</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>42.9a±1.76</td>
<td>43.5c±1.80</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>43.25c±1.06</td>
<td>43.0b±0.90</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>43.75b±1.80</td>
<td>44.0c±1.95</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>44.0a±1.65</td>
<td>44.50c±1.70</td>
<td></td>
</tr>
<tr>
<td>P- Value</td>
<td>0.044</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

Mean in each column with different superscripts were significantly different at p<0.05.

NB: A= Negative Controll (No diabetes), B= Positive Controll (Diabetic induced), C= Diabetes with Chirata (125 mg/ kg) and D= Diabetes with Metformin (150mg /kg).

Finally, the result of blood glucose levels in Group A, B, C, and D was 5.2±1.13 mmol /L, 12.15±0.49 mmol/L, 09.45±0.64 mmol, and 8.25±0.35 mmol/L respectively. The creatinine level in Group A, B, C and D were 0.85±0.07 mg/dL, 1.15±0.07 mg/dL, 0.95±0.08 mg/dL and 0.9±0.14 mg/dL respectively. The SGPT level in Group A, B, C, and D was 19±1.41 U/L, 27.44±2.82 U/L, 26.5±2.12 U/L, and 20.5±2.12 U/L respectively. The SGOT level in Group A, B, C, and D was 314±2.82 U/L, 144±3.53 U/L, 390±1.41 U/L, and 178±2.83 U/L respectively.

Table 2 Value of blood glucose, serum creatinin, SGPT and SGOT in experimental mice on 30th and 46th days of age

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBS (Blood glucose level) m mol/L</td>
<td>5.2a±1.13</td>
<td>12.15b±0.49</td>
<td>9.45c±0.63</td>
<td>8.25c±0.35</td>
<td>0.04</td>
</tr>
<tr>
<td>Creatinin(mg/dl)</td>
<td>0.85a±0.07</td>
<td>1.15c±0.07</td>
<td>0.95b±0.07</td>
<td>0.90b±0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>SGPT(U/L)</td>
<td>19a±1.41</td>
<td>27.44c±2.83</td>
<td>26.50c±2.12</td>
<td>20.50b±2.12</td>
<td>0.03</td>
</tr>
<tr>
<td>SGOT(g/dl)</td>
<td>30b±2.83</td>
<td>90a±3.53</td>
<td>70c±1.41</td>
<td>45b±2.83</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mean in each column with different superscripts were significantly different at p<0.05.

NB: A= Negative Controll (No diabetes), B= Positive Controll (Diabetic induced), C= Diabetes with Chirata (125 mg/ kg) and D= Diabetes with Metformin (150mg /kg)

4. Discussion

The experiment was performed to see the anti-diabetic efficacy of aqueous extract of chirata in alloxen-induced diabetic mice.
4.1. Body Weight

The research showed that the final body weight of positive control (diabetes induction) Group B (43.0±0.90) was significantly decreased from the treatment of negative control Group A (43.5±1.80) and similarly followed by treatment with a synthetic anti-diabetic agent such as metformin group D (44.5±1.70) and herbal drugs as chirata treatment Group C (44.0±1.95).

In the present study, it was found that the final body weight of negative control (No diabetes) and different anti-diabetic treatment groups was significantly increased (P<0.05) from the positive control groups (Diabetes) which showed similarities with the findings of [18]. They found that the body weight was significantly increased in the chirata-treated mice. This weight gain increase due to eating normal food but other groups’ body weight decreases due to necrosis of the beta cell of the liver for the induction of diabetes with the help of alloxan monohydrate. But after giving treatment to the C and D groups with the chirata extraction and metformin respectively, the body weight increased gradually due to the improvement of the diabetes level. On the other hand, there is decreasing the body weight in the diabetic control group (Group B) due to there being no given medication. These findings were also confirmed upon examination of the extraction of chirata leaf have the lowering blood glucose level in the Swiss albino mice model. This study also showed similarity with the findings of [19], who reported that daily treatment with the plant of chirata extract and rograthistaniculata supplement at 200mg/kg body weight to diabetic groups. The animal resulted in significant increases in body weight at the end of the treatment compared to diabetic groups. The study revealed a 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 [10]. Who reported that the decrease in the body weight by alloxan induced in diabetic rats was possible due to catabolism of protein and fats even though food intake was more in diabetic rats than control.

4.2. Blood Glucose Level

The research showed that the blood glucose level was significantly decreased in the negative control (No diabetes) in Group A (5.2±1.13), herbal agent treatment such as chirata extract for Group C (9.4±0.64), and synthetic medication with metformin for Group D (8.25±0.35) from the positive control (diabetes without any medication either synthetic or herbal agents) Group B (12.15±0.49).

This result also showed that the blood glucose levels were significantly (P<0.05) decreased in the treatment of chirata extract and metformin treatment groups. In the present study, it was found that the final blood glucose level of negative control (No diabetes) and different anti-diabetic treatment groups was significantly decreased from the positive control groups (Diabetes) which showed similarities with the findings of [18]. They found that the blood glucose level was significantly decreased in the chirata and metformin-treated mice.

The aqueous extract of swerchia chirata has a significant effect on blood glucose lowering activity. In the group treated with aqueous extract of chirata (Group C), their plasma glucose level is significantly decreased which is comparable with the group of metformin treatment Group D. It is proved that the aqueous extract of swerchia chirata increase the stimulation of the pancreatic beta cell of the Islets of Langerhans to secrete the plasma insulin. Finally, insulin causes the lowering of plasma blood glucose levels. Contrary to the metformin treatment group (Group D) mice show a significant decrease the plasma glucose level. The result of the antihyperglycemic effect of the aqueous extract of swertia chirata 125mg/kg body weight in this research is more or less similar to the results reported by [2]. The results of the plasma glucose level of this research of the metformin treatment group are similar to the previous report by [12]. This result is also more or less similar to the previous report by [10] Chirata (Swertia chirata) contains swerchirin, which is a potent antihyperglycemic compound [4]. It is probable that the hexane fraction of chirata contains more active compounds which showed a hypoglycemic effect. Hexane fraction of chirata induced a significant fall in blood glucose in rats compared to that of aqueous extracts of chirata. Aqueous extract of chirata at the rate of 50 mg/kg body wt. was found better dose. A similar result was reported by [17].

4.3. Creatinine Level

The present study showed that the creatinine level was significantly decreased in the negative control (no diabetes) Group A (0.85±0.07) treatment with the extract of chirata Group C (0.95±0.07) and metformin treatment Group D (0.90±0.14) from the positive control (diabetes induction) Group B (1.15±0.07).

This result showed that the blood creatinine level was significantly (p< 0.05) decreased in the treatment of herbal drugs such as chirata treatment and synthetic drugs such as metformin treatment groups compared to the other treatment.
groups. The results of this research are more or less similar to the previous report by [12]. This result is also more or less similar to the previous report by [10].

**4.4. SGPT and SGOT**

The research showed that the SGPT level was significantly decreased in negative control Group (no diabetes) A (19±1.41), chirata treatment Group C (26.5±2.12), and metformin treatment Group D (20.5±2.12) from the positive control Group B (27.44±2.83).

The Experiment also showed that the SGOT level was significantly increased in metformin treatment in Group D (45±2.83), herbal medication in Group C (70±1.41), negative control (no diabetes) in Group A (30±2.83) from the positive control (diabetes) group B (90.5±3.54).

The result is more or less similar to that of the result of the Assay of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) can provide the physician with valuable diagnostic and prognostic clinical evidence of acute hepatic and cardiac disease [8]. The much higher SGPT activity in the diabetic control compared with the normal control observed in this study complies with that of [12]. Who found a close association between SGPT activity and type II diabetes. The higher SGOT activity due to glibenclamide itself is difficult to explain because these treatments efficiently lowered blood glucose levels in diabetic rats. The significantly much higher SGPT activity without any effect on SGOT activity in the diabetic control in relation to the normal control group suggests that the rat liver and not the heart might have been affected by STZ induction. However, higher SGOT activity is reported to be associated with the later development of diabetes [13].

**5. Conclusion**

The hypothesis of obtaining plant-based medicine is beneficial to human health. Based on the in vivo experimental study and the active profile exposed through various biochemical parameters, it can be concluded that chirata extract showed significant anti-diabetic activity. Further investigations on the isolation and identification of bioactive components in the plant would help to ascertain its potency. Data from this study shows that metformin can reduce blood glucose in diabetic rats and contribute to increased body weight. Since obesity is one of the key factors that contribute to the development of Type-2 DM. Finally, the research has become successful due to lowering blood glucose level of chirata extract in diabetic conditions, improving the body weight of swiss albino mice those are treated with the chirata extraction, but also improving the blood SGPT and SGOT levels in treated mice. In the future, we can suggest that chirata extraction can also be used against the anti-inflammatory conditions, antipyretic, and IBS (Irritant Bowel Syndrome) disease.

**Compliance with ethical standards**

**Acknowledgments**

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**Disclosure of conflict of interest**

Authors declare there is no conflict of interest in publishing the article.

**Statement of ethical approval**

The present research work does not contain any studies performed on animals/humans subjects by any of the authors’.

**References**


