Comparative Efficacy of Combined Formulation of Andrographis paniculata, Lagerstroemia speciosa and Tribulus terrestris with Glimepiride on Renal and Pancreatic Injury in Alloxan Induced Type-1 Diabetic Mice


ABSTRACT

A growing number of countries around the world are using medicinal plants to treat various illnesses, including diabetes. This study was designed to elucidate the comparative efficacy of the combination of three herbal formulations Andrographis paniculata, Lagerstroemia speciosa and Tribulus terrestris on glucose intolerance, lipid profile, renal and pancreatic injury in type-1 diabetic mice induced by alloxan. A total of six groups of five Swiss Albino mice aged four weeks were studied. To assess toxicity, Group-X was given saline water, while Group-Y was given a high concentration (1 g/kg bwt) of combination herbal formulation. In normal healthy mice, there was no negative effect of the combined formula on body weight or blood glucose levels. The other four groups were labeled as Group-A, healthy normal mice; Group-B, Diabetic mice; Group-C, Diabetic mice treated with Andrographis paniculata, Tribulus terrestris 200 mg/kg and Lagerstroemia speciosa 0.5ml/animal; and Group-D, Diabetic mice treated with Amaryll® 800 g/kg bwt. After 8 weeks of treatment, the combination formulation significantly enhanced body weight loss (P<0.001) compared to the diabetic control group. When compared to diabetic control, the combined formulation and Amaryll® lowered plasma triglyceride, creatinine, and total cholesterol in a non-significant manner. However, the therapy had no effect on HDL or LDL levels. In line with these results, histological analysis showed that the combined formulation partially reduced tubular damage, pancreatic β-cell damage, and renal glomerular sclerosis and hypertrophy, reflecting its renal and pancreatic damage curative property. The obtained results suggest that combined use of Andrographis paniculata, Lagerstroemia speciosa and Tribulus terrestris as a herbal antidiabetic formulation enhances its therapeutic effectiveness in the management of hyperglycemia and diabetes-related consequences, particularly renal and pancreatic impairment.

Keywords: Andrographis paniculata, blood glucose, diabetic mice, Lagerstroemia speciosa, lipid profile, renal and pancreatic injury, Tribulus terrestris.

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I. INTRODUCTION

The most prevalent endocrine problem in human and pet animals is diabetes mellitus, a diverse syndrome rather than a single disease entity which have hyperglycemia as the hallmark results from abnormalities in insulin secretion, or action. Diabetic symptoms include polydipsia, polyuria, lethargy, polyphagia (increased hunger), tiredness, weight loss, blurred vision, balanitis (genital candidiasis), preference for sweet foods, impatience, etc. [1].

Diabetes mellitus can be classified into three primary categories (DM). Type-1 DM, previously referred to as "Insulin-Dependent Diabetes Mellitus" (IDDM) or "Juvenile Diabetes". Insulin resistance causes type-2 diabetes mellitus, often known as "adult-onset diabetes" or Non Insulin-Dependent Diabetes Mellitus (NIDDM). Gestational diabetes, the third major type, develops in pregnant women who have high blood sugar levels without having been previously diagnosed with diabetes.

Herbal treatments for unresolved chronic illnesses have gained importance in recent years due to their potency, lack of side effects, and relative affordability [2]. Synergistic activity may be created by poly herbal therapy or combination of different plants which are useful against different diseases and also for diabetes mellitus [3]. More than 1,200 species of plants with hypoglycemic potential have been discovered via ethnomedical research for traditional herbal diabetes treatment used around the world [4].

Compared to chemical medicine, traditional herbal medicinal plants have no side effect, that’s why its popularity is growing very fast. Precisely, we may have the advance technology now and modern medicines, however, we can’t alter the popularity of traditional medicines. Herbal medicine is a bit slow in treatment but it cures disease from the root [5]. When choosing between herbal medicine and chemical medicines, it is quite obvious that synthetic medications frequently fail to treat illnesses and often have much more adverse consequences [6].

Our earlier research showed that after 8 weeks of treatment, the combination of Tribulus terrestris and Andrographis paniculata considerably reduced blood glucose levels compared to the diabetic control group and enhanced body weight loss in diabetic rats [7]. In comparison to diabetes control, the combination formulation reduced plasma triglyceride and total cholesterol while leaving HDL and LDL levels unaltered. Histopathological analysis showed that the combination formulation improved pancreatic beta-cell damage, tubular damage, and renal glomerular sclerosis and hypertrophy to some extent [7]. According to the previous research, Andrographis paniculata has antipyretic, analgesic, anesthetic, anti-inflammatory, anti-infective, anti-neoplastic, and immunosuppressive activities [8]. So far, there are no strong arguments to support its combined effectiveness in vivo. So, the purpose of this work was to clarify A. paniculata’s potential as an antihyperglycemic agent in alloxan-induced diabetic mice. On the other hand, studies have shown that saponins in the extracts of Tribulus species exhibited hypoglycemic and hypolipidemic effects in diabetic rats [9]. Tribulus terrestris significantly reduced the level of serum glucose, serum triglyceride, and serum cholesterol in alloxan induced diabetic mice [10]. Lagerstroemia speciosa (L) or Banaba is locally known as ‘Jarul’ in Bangladesh. Corosolic acid, an active ingredient in these extracts, displays a potential anti-diabetic activity [11], as well as anti-oxidant, anti-inflammatory and antihypertension properties [12]. The leaves of this tropical plant have been utilized by tribal people as a folk remedy for heart disease, diabetes, and renal diseases [13].

The need for an alternative natural treatment for diabetes mellitus is critical because the anti-diabetic medications that are currently available in Bangladesh are primarily imported, expensive, and also build resistance to diabetes with numerous negative effects. Considering all three constraints, in this experiment, we were interested in developing an indigenous medical system (herbal therapy) as an alternative to chemical antidiabetic medications. This pioneer work has been undertaken with the following aims to evaluate whether Tribulus terrestris, Andrographis paniculata and Lagerstroemia speciosa have any adverse effect in healthy mice and beneficial effects in alloxan induced type-1 diabetic mice. Hence, a comparative study was undertaken between the effects of combined herbal formulation of these three medicines and commercial oral hypoglycemic drug (Amaryl®).

II. MATERIALS AND METHODS

This research was carried out at the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh to assess the efficacy of combined formulation of Andrographis paniculata, Lagerstroemia speciosa and Tribulus terrestris with Amaryl® on glucose intolerance, renal and pancreatic damage on male white albino mice (Mus musculus), where diabetes was induced by alloxan.

A. Collection of Mice

Thirty (30) healthy adult male Swiss Albino mice were collected from the International Centre for Diarrhoeal Diseases Research and Rehabilitation, Bangladesh (ICDDR, B), Mohakhali, Dhaka, to test the antidiabetic effect of the formula and extract. Before starting the experiment, two weeks time was given to all mice for acclimatization to the new environmental conditions (temperature at 28 ± 2°C and a relative humidity of 70-80%, 12-hour light and 12-hour dark cycle). They were fed with standard pellet and water ad libitum. The mice were divided into six equal groups at random and housed in compartmentalized rectangular metallic cages (9×11×7 cubic inches) wrapped in wire mesh with a maximum of five mice per compartment. Each animal's normal body weight and blood glucose level were determined using an electric balance (Camry, EK3052) and a glucometer (Omron, E-OHS-BD), respectively.

B. Experimental Procedure

Prior to examination on diabetic animal, ten normal mice were divided into two groups (5 mice in each group), marked as study-1 and maintained as follows: Group X served as saline treated healthy control group and Group Y mice was fed combined formulation of Andrographis paniculata and Tribulus terrestris and extract of Lagerstroemia speciosa at dose rate of 1g/kg body weight for 7 days to assess the impact of formula and extract on normal mice. Then, in study-2, 20 mice were divided into four groups containing 5 mice in each

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group and used to assess the effectiveness of *Andrographis paniculata*, *Lagerstroemia speciosa* and *Tribulus terrestris* on type-1 diabetic mice induced by alloxan. Group A: oral saline water was given to mice, nourished with a normal diet, and water *ad libitum* (named normal control group); Group B: diabetes was induced in mice by intraperitoneal injection of alloxan monohydrate injection (SIGMA-ALDRICH Company, UK) 120 mg/kg; (named diabetic control group); Group C: mice received alloxan monohydrate injection for confirming diabetes. Then to observe the effectiveness of the formulation as antidiabetic drug, *A. paniculata* and *T. terrestris* (200 mg/kg body wt. and aqueous extract of *L. speciosa* at (0.5 ml/animal) were supplied for 08 weeks, (named Diabetic + Formula + Extract group) and Group D: mice received alloxan monohydrate injection for confirming diabetes. After that tablet Amaryl® was administered orally 800 µg/kg body wt. for 08 weeks. Body weight and blood glucose were recorded on Day 0 (Pre-treatment) and weeks 2, 4 and 8 (during treatment). This group was named as Diabetic + Amaryl® group to compare the effectiveness of combined formulation with commercially available drugs against diabetes. Body weight and fasting blood glucose level of each mouse were measured after 18 hours of fasting before alloxan monohydrate injection, then 15th day of alloxan monohydrate injection. Furthermore, body weight and fasting blood glucose level of each mouse were assessed on day 0 (Pre-treatment) and weeks 2, 4 and 8 (during treatment), respectively.

C. Collection, Preservation and Administration of Combined Formula, Extract and Amaryl® Tablet

The combined formulation of *Andrographis paniculata*, *Lagerstroemia speciosa* and *Tribulus terrestris* was prepared by Kabiraz Kazi Shazzad Hossain, the proprietor of Janani Chikitsalaya, Barisal, Bangladesh and got as a generous gift. Due to its patent right, the formulation of the three plants ingredient was undisclosed. With the assistance of Kabiraz Kazi Shazzad Hossain, the plants were verified as real, and the voucher samples were kept in the Department of Pharmacology, BAU. The formulation was administered at a dose of 200 mg/kg body weight mixing with water. The aqueous extract of *L. speciosa* was prepared by collecting it from Garden of Bangladesh Agricultural University, Mymensingh, and then grinded using mortar and pestle by mixing with 3 mL of water and administered at a dose of 0.5 mL/animal by oral gavaging for 8 weeks. The glimepiride tablet (Amaryl®) was collected from K.R. Market, Bangladesh Agricultural University, Mymensingh and administered 800 µg/kg bwt by oral gavaging for 8 weeks.

D. Analysis of Blood Glucose and Lipid Profiles

Blood samples were collected from tip of the tail on week 0 (pre-treatment), 3, and 8 (during treatment period) for estimation of blood glucose using a glucometer. On the 8th week of experiment, animals were given rest overnight, and then on the next day they were sacrificed by cervical dislocation under anesthesia with sodium pentobarbital (65 mg/kg, i.p.). Following surgically opening the thoracic and abdominal cavities, blood was drawn straight from the heart using a sterile syringe and needle. For hematological analyses and the collection of plasma, approximately 1mL of blood from the syringe was placed in the test tube containing anticoagulant (3.8% Na citrate solution). In order to separate the plasma, it was centrifuged at 4500 rpm for 10 minutes, and then it was placed in storage at -20 °C temperature for further analysis.

Plasma total cholesterol (TC) and triglyceride (TG) levels were measured spectrophotometrically with reagent (Linear Chemicals, S.L., Barcelona, Spain), and plasma creatinine was measured biochemically. The plasma TG level was determined using a kit from Linear Chemicals, S.L., Barcelona, Spain. The absorbance was measured at 500 nm. The value was given in milligrams per deciliter (mg/dL).

E. Histopathological Study of Kidney and Pancreas

The mice were fasted overnight at the end of the treatment; however, they were still given free access to water. They were then given anesthesia with sodium pentobarbital (65 mg/kg, i.p.) and sacrificed. After sacrificing the animals, kidney’s perfusion was done with an isotonic saline for the removal of the blood. Kidney and pancreas of the animal was removed surgically and immediately blotted using filter paper to remove traces of blood. Thereafter, the tissues were suspended in 10% formal saline for fixation preparatory to histological processing. Light microscopic analysis was performed on multiple tissue sections from each organ in each group, as well as images representative of the typical histological profile [14]. Pancreatic tissue samples were fixed in 10% buffered neutral formalin, embedded in paraffin, sectioned at 5µm and stained with Hematoxylin & Eosin [15] to detect the pancreatic islets. Using a microscope eyepiece connected to a computer monitor, photomicrographs were taken, and observations were made [16].

F. Statistical Analysis

The data were analyzed using SPSS software version 20 (SPSS INC. Chicago, IL, USA). The student t-test (Unpaired) in the Graphpad Prism program was used to assess differences between the animal groups in Study-1 whereas the data from Study-2 were statistically analyzed using one-way ANOVA, followed by Post-hoc Bonferroni tests, and expressed as mean ± standard deviation. The statistical significance level was set at P<0.05, which is considered significant in contrast to the control and standard.

III. RESULTS AND DISCUSSION

A. Toxicity Study of the Formula and Extracts

In order to determine whether there was any negative or toxic reaction compared to an animal given saline as a control, a high dose (1 g/kg) of the combination formulation of *T. terrestris*, *A. paniculata*, and extract of *L. speciosa* was administered. The results showed that between day 0 and day 7, in mice fed either saline water or a formulation, there was no discernible variation in body weight or blood glucose levels (Fig. 1). This indicates that the newly developed herbal formulation has no adverse effects on the body weight and blood glucose levels of healthy mice. Throughout the investigation period, no unusual behaviour was seen. Also, previous to this therapy, the groups had the same basal body weights.
B. Formula and Extract Improved Body Weight in Diabetic Mice

When we induced diabetes using alloxan at a dose of 120 mg/kg, it was observed that alloxan dramatically reduced body weight in mice (Fig. 2A). Amaryl® treatment increased significantly body weight (P<0.001) which is higher than combined formulation group compared to diabetic control group (Fig. 2A). When compared to the mice’s respective diabetic control group, daily treatment with formula and extract significantly (P<0.001) increased the decrease in body weight in alloxan-induced diabetes mice. Formula and extract increased body weight (P<0.01) but treatment with Amaryl® 800 µg/kg bwt improved significant body weight gain (P<0.001) when compared to diabetic control group. A significant reduction in body weight was a hallmark of alloxan-induced diabetes, which was also compatible with Erejuwa et al findings [17]. This loss or degradation of structural proteins is what causes the body weight to decrease, as structural proteins are known to contribute to body weight. Administration of the combination formulation during the duration of the 8-week treatment period considerably improved body weight loss in the alloxan-induced diabetes mice as compared to diabetic control animals. This result supports that made by Weitgasser et al. [18], who discovered that Amaryl® has the ability to neutralize body weight, stating that treatment with Glimepiride causes a considerable and stable reduction of weight compared to baseline. Diabetes control animals experienced consistent weight loss over four weeks [19]. Then, when given ethanol leaf extract of A. paniculata (250 mg/kg and 500 mg/kg) with glibenclamide, body weight of diabetic rats was considerably (P<0.01) increased compared to diabetic control.

C. Formula and Extract Lowered Blood Glucose Level in Diabetic Mice

The groups had comparable basal blood glucose levels prior to treatment. A consistent overdose of formula and extract for 7 days could not show any consequence on blood glucose levels in normal healthy mice. Blood glucose level was substantially increased in mice with alloxan injection 120 mg/kg body weight in group B, C and D. 8 weeks treatment of mice in group C and D with both combined formulation and Amaryl®, respectively, showed a reduced blood glucose level (Fig. 2B). Compared to diabetic control group, a significant (P<0.001) low blood glucose level was observed in group C and D, with daily dosing of formula and extract combination and Amaryl®, respectively. Between the combination formulation and Amaryl® treated groups, there was no discernible difference. The dose and route of administration of alloxan monohydrate was followed as described elsewhere [20]. Amaryl® was found to lower blood glucose level significantly (P<0.01) in diabetic control rats [19]. The cause behind this is glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic β-cells.

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Fig. 1. Effects of formula and extract on (A) body weight and (B) blood glucose (Mean±SEM) in normal healthy mice (n=5).

Fig. 2. Effects of formula and extract on (A) body weight and (B) blood glucose in alloxan induced type-1 diabetic mice (n=5).

*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) compared with normal control Vs diabetic control and diabetic control Vs treatments.
Traditional plants may be a potential source for novel hypoglycemic substances as there is an increasing tendency toward using natural therapies in alongside conventional medication [21]. Treatment with *A. paniculata*, *L. speciosa* and *T. terrestris*, showed a significant (P<0.01) decrease in the blood glucose level. This result is in conformity with others [19], [22]–[24]. Saponin from *Tribulus terrestris* significantly reduced serum glucose level by 26.25% and 40.67% in normal and diabetic mice, respectively [5]. According to earlier studies, giving diabetic rats 50 mg/kg of *T. terrestris* alcoholic extract after 2, 4, and 6 hours of treatment resulted in considerably lower blood glucose levels than giving the animals no treatment [9]. High-fat and high-fructose diet fed rats were shown to significantly (P<0.05) decrease pre- and post-prandial blood glucose level after a five-day treatment with *A. paniculata* extract [25].

D. Effects of Formula and Extract on Lipid Profile and Plasma Creatinine in Diabetic Mice

Compared to normal control group (98±1.15 mg/dL), alloxan injection resulted in a significant increase (P<0.01) in total cholesterol (TC) level in diabetic control mice (134.33±5.46 mg/dL). TC level of mice restored the parameters levels to normal values after daily dosing with formula and extract for 8 weeks (Fig. 3A). Administration of formula, extract and Amaryl® was also found to restore the LDL (Fig. 3B) and HDL (Fig. 3C) parameters levels to normal values. The plasma triglyceride (TG) level increased significantly (P<0.01) in diabetic control compared to normal control group (Fig. 3D). However, a non-significant restoration of this parameter was observed when herbal drugs and the standard drug Amaryl® were compared. Also, oral administration of the formula's extract demonstrated to return plasma triglycerides to normal status, while Amaryl® considerably lowers creatinine levels (P<0.05) when compared to the diabetic control group (Fig. 3E).

The groups' baseline total cholesterol levels were comparable before the treatment. During an 8-week treatment period, administration of the combined formulation and Amaryl® to alloxan-induced diabetic mice resulted in non-significantly lower total cholesterol levels. This result is partially similar with Chhatre et al. who found that the aqueous extract of *Tribulus terrestris* decreased cholesterol-induced hyperlipidemia, with a decrease in cholesterol level in blood [26]. The hypolipidemic effect of *A. paniculata* was also observed by others [27]. In alloxan induced diabetes model HDL and LDL levels were increased in diabetic control group compared to control mice. After treating the diabetic mice with combined formulation and Amaryl®, HDL and LDL cholesterol values were decreased but not statistically significant.

![Fig. 3](image-url) Effects of formula and extract on plasma (A) total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol, (D) triglyceride, and (E) creatinine in alloxan induced type-1 diabetic mice (n=5).
Plasma triglyceride values were increased in diabetic control (DC) group significantly (P < 0.01) compared to control (C) group. After treating them with combination of formula and Extract, also Amaryl®, all of them decreased the triglyceride values non-significantly. In comparison to normal rats, Nugroho et al. estimated that Andrographis has a mild lowering effect on triglyceride levels (P < 0.05) [25]. The plasma creatinine level in combined formulation treated group was decreased non-significantly and in Amaryl® treated group the creatinine values decreased significantly (P < 0.01). Premananth et al. (2015) found that diabetic control rats had higher serum creatinine, urinary creatinine, and serum urea levels than normal rats, which were significantly reduced by glibenclamide and the extract after 28 days of treatment [19].

E. Renal and Pancreatic Protective Effects of Formula and Extract in Diabetic Mice

To determine the effect of the formulation on diabetic nephropathy and pancreatic injury, paraffin embedded sections of renal cortex and pancreatic tissue were stained with hematoxylin and eosin stain. In normal mice, the kidney cortex appeared normal, whereas, in diabetic control mice most glomeruli exhibited interstitial fibrosis, tubular necrosis, glomerular hypertrophy, capillary occlusion (Fig. 4A, 4B). In addition, many cortical tubules were vacuolated. All these damages were partially improved in diabetic mice treated with Andrographis paniculata, Tribulus terrestris and Lagerstroemia speciosa extract (Fig. 4C). Diabetes was associated with an increase in Glomerulosclerotic index (GSI) compared to diabetic control mice. GSI was lowered in diabetic mice treated with combined herbal formula and extract compared to glimepiride. This finding is partly supported by an earlier research who observed the renal damage protective effect of A. paniculata in diabetic mice [28].

![Fig. 4. Photomicrographs of mice kidney (arrows show renal glomerular damage and inflammation) stained by H & E stain of (A) normal mice, (B) diabetic mice, (C) combined formula and extract, and (D) effects of Amaryl® (800 µg/kg bwt). Microscopic magnification (20X).](image)

Pancreatic section from the control mice exhibited normal architecture of endocrine portion with normal islets of langerhans cell (Fig. 5A). Lymphocytic infiltrations, atrophy, and beta cell destruction were observed in diabetic mice (Fig. 5B). This group's islet cells were small and shrunken. The pancreas of diabetic mice treated with the combined formulation 200 mg/kg and extract 0.5 ml/animal demonstrates a rise in the amount of islet cells (Fig. 5C). In diabetic mice treated with glimepiride, hematoxylin and eosin sections of the pancreas revealed partial regeneration of β-cells (Fig. 5D). Similar results were found in diabetic rats with Tribul treatment [9]. To the best of our knowledge this study was performed for the first time to find out the renoprotective effect of Andrographis paniculata, Tribulus terrestris and Lagerstroemia speciosa extract in diabetic mice. Therefore, due to limited review and literature we are unable to compare our present findings with others elsewhere.

IV. Conclusion

Based on recent experimental evidence, it is possible to infer that the newly developed herbal combination formula has a beneficial impact on renal and pancreatic injury, glucose intolerance, and type 1 diabetic mice produced by alloxan. The findings of the present study are partially or in some cases completely compatible to antidiabetic market preparation Glimepiride. In conclusion, this investigation confirms herbal physicians' longstanding use of the herbal medicine for diabetes treatment. A. paniculata, T. terrestris, and L. speciosa together may offer up a new treatment path against diabetes and its consequences. More in-depth investigations are advised in order to comprehend the mechanism of action and determine whether this type of combination formulation has higher anti-diabetic efficacy.

AUTHORS CONTRIBUTION

PH, ASMEI, KNK, KNK, MFF: sample collection, analysis. PH, FA, SRD, MIH, AH: manuscript writing and revision; KR, NS, FA: planning, conceptualization, supervise and revised the manuscript; KR, KNK, PH, ASMEI, MFF: Sample analysis and data compilation. All authors have read and approved the final manuscript.
ETHICS APPROVAL
All experimental procedures in this original project were performed according to the guidelines for the care and use of animals as described by Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University (BAU), Mymensingh-2202 [Approval number: AWEEC/BAU/2017(18)].

DATA AVAILABILITY
Due to important public health and general public issues, the datasets created during and/or analyzed during the current investigation are not publicly available, however, they are available from the corresponding author upon justifiable request.

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CONFICT OF INTEREST
The authors declare no relevant financial or non-financial conflict of interest to disclose.

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