The study aims to evaluate the effect of *Tribulus terrestris* and *Andrographis paniculata* on body weight, blood glucose, lipid profile and renal protective capability in alloxan induced type-1 diabetic mice. Six groups of five Swiss Albino mice each, at four weeks of age were used in this experiment. Group-X were fed saline water and Group-Y with combined herbal formulation at a high dose (1 g/kg bwt) to assess toxicity. The remaining four groups were designated into healthy control, diabetic control, diabetic + Amaryl® 800 µg/kg, Diabetic + Formulation 200 mg/kg bwt. The combined formulation improved body weight loss and lowered blood glucose levels significantly ($P<0.001$) compared to diabetic control group after 8 weeks of treatment. The total cholesterol and plasma triglyceride were decreased at a significant level ($P<0.01$) compared to diabetic control. However, High density lipoproteins (HDL) and Low density lipoproteins (LDL) levels remained unchanged by combined formulation compared to diabetic control. The combined formulation also lowered the plasma creatinine levels in the formulation treated group compared to diabetic control. Histo-pathological evaluation revealed that combined formulation partially improved renal glomerular sclerosis and hypertrophy, tubular damage and pancreatic β-cells damage. The combined herbal formulation may have antidiabetic and renal protective capability in alloxan induced type-1 diabetic mice.

**Key words:** *Tribulus terrestris*, *Andrographis paniculata*, blood glucose, lipid profile, renal injury, antidiabetic effects.

**INTRODUCTION**

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both (Mutalik et al., 2003; Joseph and Jini, 2011). With an estimated 371 million affected adult people worldwide, diabetes mellitus is one of the most widespread diseases that caused 4.8 million deaths in 2012 (IDF, 2013). The most prevalent form globally is the non insulin dependent diabetes mellitus (NIDDM type-2) which is associated...
with elevated post-prandial hyperglycemia. The classic symptoms of untreated diabetes are weight loss, polyuria, polydipsia and polyphagia (Cooke and Plotnick, 2008). Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes (Kitabchi et al., 2009). Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in diabetic patients (Xie et al., 2003). Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (Bohannon, 2002). The base of healthcare system has been founded by medicinal plants worldwide since the primitive stage of humanity and plant products are still a major source of drug/formulation for the treatment of various diseases (Kamboj, 2000). The active principles present in medicinal plants have been reported to possess various activities such as pancreatic β-cell regeneration, stimulating insulin release from β-cells, showing insulin-like action, fighting the problem of insulin resistance, and reducing the uptake, absorption and utilization of glucose (Wadkar et al., 2008).

*Tribulus terrestris* L, is commonly known as puncture vine, caltop, yellow vine, goat head and devil’s horn. Saponins in the extracts of *Tribulus* species showed hypoglycemic and hypolipidemic effects in diabetic rats (El-Tantawy and Hassanin, 2007). *T. terrestris* significantly reduced the level of serum glucose, serum triglyceride and serum cholesterol. (Chhatre et al., 2008). The decoction of *T. terrestris* showed inhibition of gluconeogenesis in mice (Amin et al., 2006). Thus, *T. terrestris* could be beneficial in the treatment of diabetes by lowering blood glucose, lipid levels, and by protecting kidney and pancreas.

*Andrographis paniculata*is plant has been effectively used in traditional Asian medicines for centuries. Andrographolide appears to dose-dependently reduce plasma glucose concentration in streptozotoc in induced diabetic rats and normal rats. The hypoglycemic effect of *A. paniculata* is due to insulin release from pancreatic β-cells through ATP-sensitive potassium channels, is similar to other insulin tropical antidiabetic agents (Wibudi et al., 2008). However, the uses of these two plants extract combinely in diabetes to ameliorate its complications such as diabetic nephropathy and pancreatic injury are not yet evaluated. This research was conducted to evaluate the effects of *Tribulus terrestris* and *A. paniculata* on blood glucose, lipid profile that is, total serum cholesterol (TC), serum triglyceride (TG), serum HDL cholesterol, serum LDL cholesterol, serum creatinine, diabetic nephropathy, and pancreatic injury in alloxan induced diabetic mice.

**MATERIALS AND METHODS**

The experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. Experimental protocols and animal care were performed according to the guidelines for the care and use of animals established and approved by the Animal Welfare and Ethical Committee, Bangladesh Agricultural University, Mymensingh.

**Collection and acclimatization of mice**

A total of 30 healthy adult male Swiss albino mice were collected from International Centre for Diarrheal Diseases Research and Rehabilitation, Bangladesh (ICDDR) Mohakhali, Dhaka. All the mice were acclimatized to the new environmental condition for a period of two weeks. The cages were kept in well ventilated room at 28± 2°C and a relative humidity of 70 to 80% with natural day and light. The normal body weight and normal fasting glucose level of each animal was measured by electric balance (Camry, EK3052) and Glucometer (Omron, E-OHS-BD), respectively.

**Experimental design**

**Study 1**

Two groups (Group-X and Group-Y) each group containing 5 mice were used, mice of Group-X were fed normal chow and administered saline water orally, and the mice of Group-Y were supplied combined formulation at a dose of 1 gm/kg body weight. The body weight and blood glucose level were calculated after 7 days. The result was taken to check whether there is any adverse effect on glucose level and body weight gain in healthy animals.

**Study 2**

After being sure about the promise of the formulation on normal mice, the 2nd step of the experiment began to evaluate the effects of combined formulation on diabetic mouse model. For this, twenty (20) mice of one month age were used in this study. The mice were randomly divided into four (4) groups, each group containing five (5) mice. The mice of Group-A were administered with saline water orally and fed normal diet. This group served as Healthy Control group. Alloxan Monohydrate injection was given at a dose rate of 120 mg/kg in intra-peritoneal route to each mouse to induce diabetes in the remaining three groups. The mice were fed normal diet and given water ad libitum. During that period at week 0, 2, 4 and 8 the body weights and blood glucose levels were measured. One group served as Diabetic Control group. Combined formulation was given orally to alloxan induced mice at a dose of 200 mg/kg body weight for 8 weeks to another group. This group served as Diabetic + Formulation to find the effect of formulation as antidiabetic drug. Tablet Amaryl® was administered at a dose rate of 800 µg/kg bwt for 8 weeks. This group served as Diabetic + Amaryl®.

**Collection of drugs and herbal combined formulation**

A bottle of 25 g Alloxan Monohydrate (SIGMA- ALDRICH Company, UK) was purchased from the market. The combined herbal formulation of *T. terrestris* and *A. paniculata* was a kind gift of Kabiraz (Unani Ayurvedic Doctor) Kazi Shazzad Hossain, Proprietor of Janani Chikitsalaya, Barishal, Bangladesh. The plants were authenticated with the help of Kabiraz Kazi Shazzad Hossain and the Voucher samples were stored in the department of Pharmacology, Bangladesh Agricultural University. The Glimepiride tablet (Amaryl®) was collected from Local Market, Bangladesh.
Collection of sample
The mice were anaesthetized under sodium pentobarbital (65 mg/kg, i.p.) anesthesia and sacrificed and blood was collected directly from the heart. Kidneys were perfused with an isotonic saline and removed with pancreas. Both kidney and pancreas were stained with Haematoxylin and Eosin (H & E) stain.

Biochemical analysis for oxidative stress
Assay of plasma lipid peroxidation
For measuring the rate of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation was used. Plasma samples were mixed with Tricarboxylic acid (TCA) (20%) and the precipitate was dispersed in H$_2$SO$_4$ (0.05 M). TBA (0.2% in sodium sulfate 2M) was added and heated for 30 min in boiling water bath as described previously (Rafiq et al., 2012). TBARS adducts were extracted by n-butanol and the absorbance was measured at 532 nm. This reaction is formed in acidic pH and high temperature, and the maximum absorption is a pink complex in 532 nm (Alam and Fareed, 2016).

Statistical analysis
All data were expressed as Mean ± SEM, and differences among the groups of animals were compared using one-way ANOVA with post-hoc Bonferroni test. The preliminary control data from study-1 was compared by using Student’s t-test.

RESULTS
Study 1
No significant difference was observed in glucose level between the groups on day 0 and day 7. This data from study-1 suggests that even in high dose herbal combination formulation have no significant effect on body weight and blood glucose level in healthy mice (Figure 1).

Study 2
After induction of diabetes in mice, there was a significant ($P<0.001$) reduction in body weight of alloxan induced type-1 diabetic mice from 32.1±1.51 g to 28.4±0.85 g. In treated groups a significant ($P<0.001$) increase in body weight was observed compared to diabetic control group, which was almost close to healthy control group (Figure 2).

In diabetic control group after alloxan administration the blood glucose levels increased to 14.42±0.9 mmol/L at week 2 which was further increased to 16.3±1.1 at week 8. As well as, combined herbal formulation powder was found to significantly ($P<0.001$) reduce blood glucose levels from 15.34±0.9 mmol/kg to 12.06±0.7 mmol/L at week 2 to week 8 respectively, comparing with the diabetic control group (Figure 3).

There was no significant changes in HDL values after treating them with Amaryl® or combined herbal formulation (200 mg/kg bwt) but formulation treatment prevent the further worsening in cholesterol level in alloxan induced diabetic mice (Figure 4). After 8 weeks of treatment, average triglyceride (TG) level of diabetic control group elevated significantly ($P<0.001$) to 221.25 ± 8.7 mg/dL. On the other hand, at week 8 of treatment, combined formulation treated group also showed lower triglyceride (TG) level 185.5±4.7 mg/dL comparing to diabetic control mice. There was no significant changes in plasma creatinine values after treating them with Amaryl® or combined herbal formulation.
Figure 2. Effect of *T. terrestris* and *A. paniculata* on body weight (g) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *Significant at 5 percent level (P<0.05); **Significant at 1 percent level (P<0.01); ***Significant at 0.1 percent level (P<0.01) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

Figure 3. Effect of *T. terrestris* and *A. paniculata* on blood glucose level (mmol/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *Significant at 5 percent level (P<0.05); **Significant at 1 percent level (P<0.01); ***Significant at 1 percent level (P<0.01) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.
Healthy Control  
Diabetic Control  
Diabetic + Amaryl 
Diabetic+ Formulation 
60 
70 
80 
90 
100 
110 
120 
130 
140 
150 

Plasma Cholesterol (mg/dL)  

Plasma Triglyceride (mg/dL)  

HDL (mg/dL)  

LDL (mg/dL)  

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Figure 4. Effect of *T. terrestris* and *A. paniculata* on lipid profile (mg/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *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 Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

formulation (200 mg/kg bwt) but formulation treatment prevent the further increase in creatinine level of alloxan induced type-1 diabetic mice (Figure 5).

Induction of diabetes with alloxan was associated with marked histological changes in the kidney over a period of 8 weeks as revealed by tubular epithelial damage, glomerulo sclerosis, glomerular atrophy, abnormal renal corpuscles, interstitial fibrosis, (B). The healthy section showed normal architecture of the renal glomerulus and tubules (A). Treatment of diabetic mice with the formulation afforded significant protection from renal damage (D) whereas tubular damage was also pronounced in mice treated with Amaryl® (C) (Figure 6). In the untreated diabetic mice atrophy and degeneration were observed mostly in the β-cells specially in the central portion of the islets of langerhans. Treatment of diabetic mice with combined formulation and Amaryl® led to normalization of the affected β-cells (Figure 7).

Alloxan induced mice showed increased plasma lipid peroxidase level indicating systemic oxidative stress. At 8 week, treatment with combined formulation decreased plasma lipid peroxidase level in diabetic mice (Figure 8).

DISCUSSION

The combined formulation of *T. terrestris* and *A. paniculata* was administered at a high dose (1 g/kg) to find out any toxic effect in comparison to saline control animal. The finding suggests that the body weight and blood glucose level did not have any significant variation between the 0 day and 7 day in saline water and formulation fed mice. It implies that the newly derived herbal formulation has no adverse effect on blood glucose level of healthy mice. The basal body weight before this treatment was similar between the groups. Alloxan @ 120 mg/kg significantly decreased the body weight in mice. Body weight was elevated by the treatment with both combined formulation and Amaryl® treatment for 8 weeks.

During 8 weeks of treatment period, administration of combined preparation in alloxan induced diabetic mice showed a significant improvement in body weight loss when compared to diabetic control mice. Alloxan induced elevated blood glucose levels were reduced by the treatment with both combined formulation and Amaryl®
Figure 5. Effect of *Tribulus terrestris* and *Andrographis paniculata* on plasma creatinine level (mg/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *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 Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

During 8 weeks of treatment period, administration of combined preparation in alloxan induced diabetic mice showed a significant improvement in HDL-cholesterol loss when compared to diabetic control mice. This result partially matched with Nugroho et al. (2014) who found that *A. paniculata* has mild lowering effect on HDL–cholesterol levels from pre to post. On the other hand, Sen et al. (2011) found that treatment with *Tribulus terrestris* significantly increased serum HDL level and decreased elevated LDL level when compare to diabetic control. Administration of combined preparation in alloxan induced diabetic mice lowered the LDL level when compared to diabetic control mice. Sen et al. (2011) also exposed the LDL lowering potential of *T. terrestris* at a level of 25.7% compared to diabetic control. Lakshmia et al. (2014) studied the LDL lowering potential of *A. paniculata* to a significant level (P<0.01) in contrast to cholesterol treated animal.

The plasma triglyceride level was significantly (P<0.01) decreased in formulation and Amaryl® treated groups in contrast to diabetic control groups. This result partially matches with the evaluation from Sen et al. (2011) describing that, elevated serum TG level was decreased in extract and glibenclamide treated group. Formulation and Amaryl® both decrease serum creatinine but was not so significant. Premanath et al. (2015) described that diabetic control mice exhibited higher plasma creatinine,
Figure 6. Photomicrographs of histopathological studies of Kidney sections of normal and experimental diabetic mice. Paraffin embedded sections of renal cortex were stained with hematoxilin and eosin (H&E). Representative light micrographs (20 X) from each mice groups are shown. (HE-20X).

Figure 7. Representative histopathological profiles on β-cells (arrow show one islet) Paraffin embedded sections of pancreatic tissue were stained with hematoxilin and eosin (H&E). Representative light micrographs (20 X) from each mice groups are shown. (HE-20X).
urinary creatinine and serum urea levels compared to those of normal mice. Combined formulation of *T. terrestris* and *A. paniculata* as well as Amaryl® significantly increased the number and size of islet cells especially in the β-cell region. Atrophy and degeneration of the β-cells of the central zone of the islets of Langerhans of untreated mice were improved in mice treated with formulation of *T. terrestris* and *A. paniculata* as well as Glimepiride.

Oxidative stress leads to the onset and subsequent complications of type 2 diabetes mellitus, neural damage, vascular dysfunction, cognitive decline and renal injury. In this regards previous study showed that a dramatic loss of learning and memory function was observed in mice with large increases in brain oxidative stress, whereas an antioxidative treatment almost completely reversed the behavioral changes (Liu et al., 2003). There is also clinical evidence of increased oxidative damage in subjects with mild cognitive impairment has been highlighted (Keller et al., 2005).

Dobrian et al. (2003) reported that high salt intake induces increased vascular oxidative stress in rats. Other clinical studies also highlighted that increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications including nephropathy (Abe et al., 2011; Goodarzi et al., 2010).

In contrast, present study demonstrated that alloxan induced mice showed argumentation of plasma lipid peroxidation. The increase in TBARS, an index of lipid peroxidation in the diabetic mice might be due to increased levels of oxygen free radicals. In animal studies, tea polyphenol administration was shown to decrease serum TBARS level due to its potential antioxidant activity (Sharifzadeh et al., 2017). Along with previous finding, these results suggest that argumentation of oxidative stress plays an important role in the pathogenesis of pancreas and renal injuries in alloxan induced diabetic mice. In addition, the mechanism of the synergistic or beneficial effects of combination preparation is not yet clear; however, these

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**Figure 8.** Effect of *T. terrestris* and *A. paniculata* on plasma Lipid peroxidation (nmol/mL) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *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 Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.
studies have highlighted the potential roles of their antioxidative properties (Abe et al., 2011; Goodarzi et al., 2010; Sharifzadeh et al., 2017).

Conclusion

These data suggested that the hypoglycemic, hypolipidemic and kidney protective potential of combined formulation of T. terrestris and A. spaniculata in alloxan induced mice may be due to their antioxidative properties. Further studies are needed to isolate the active ingredients in combination formulation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author’s sincere thanks go to the Research Support and Publications Division, University Grants Commission, Dhaka, Bangladesh (UGC) for funding the study. Tribulus terrestris and Andrographis paniculata combination formulation was a kind gift from Kabiraz Kazi Shazzad Hossain, Proprietor of Janani Chikitsalaya, Barishal, Bangladesh.

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