

Protective Effect Of Hydro-Ethanollic Extract Of Bushweed Flueggea Virosa On Renal Damage In Streptozotocin-Induced Hyperglycemia In Rat

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Abstract

Aim: To study the effect of hydro-ethanollic extract of Flueggea virosa on Streptozotocin-induced **hyperglycemia** in rats with respect to kidney function parameters and histology.

Methods:

Daily single oral doses of Flueggea virosa extract (100 and 200 mg/kg) and the anti-diabetic drug Metformin (500 mg/kg) were administered for 21 days to three groups of rats rendered diabetic by a single injection of Streptozotocin (60mg/kg). The body weight and blood glucose levels were recorded every 7th day. On the 21st day, the rats were euthanized and blood collected for testing kidney functions.

Results: Treatment of Flueggea virosa extract and metformin significantly reduced fasting blood glucose levels, blood urea, creatinine, uric acid, blood urea nitrogen and increased the levels of total protein. Histological studies showed metformin and hydro-ethanollic extract of Flueggea virosa retard the onset of glomerular hypertrophy, mesangial expansion, tubular dilation, collagen fiber deposition and inflammatory cells accumulation. Thus indicated delayed effect of hydro-ethanollic extract of Flueggea virosa on progression of diabetic nephropathy.

Conclusion: Rats rendered diabetic by Streptozotocin, were administered hydro-ethanollic extract of Flueggea virosa (200 mg/kg) and metformin. These treatments reduced hyperglycemia and progression of diabetic nephropathy.

Keywords: Wistar Albino rats, hydroethanollic extract of Flueggea virosa, Metformin, Streptozotocin-induced hyperglycaemia, hypoglycemia, nephropathy.

Significant findings of the study

Significant reduction in blood glucose, increase in body weight of treated diabetic rats, decrease in blood sugar level and improved kidney functioning as revealed in histological and biochemical studies indicated renoprotective and hypoglycemic effects of a hydro-ethanollic extract of Flueggea virosa and metformin.

Introduction

Diabetes has recently emerged as one of the world's leading metabolic diseases, causing significant morbidity and mortality. Obesity, sedentary lifestyle, diet, family history, insulin resistance, age, and lack of exercise are all contributing factors [1]. The disorder and its treatment are major contributors to the nation's high economic loss [2]. Herbal anti-diabetic medicines have gained popularity in both developed and developing countries due to their natural availability, lack of side effects, and, most importantly, low cost.

Herbs and herbal products have been widely studied as complementary and alternative medicines over the last few decades. Natural products have already been shown to be a potential source for the treatment of many diseases, but they frequently lack scientific validation and data to back them up. Due to a lack of scientific data, the World Health Organization (WHO) has placed a premium on scientific evaluation of the efficacy of plant-based drugs [Ref A]. There is a lot of interest in the search for plant-based remedies with antiglycation activity. This is because these remedies have the potential to inhibit the formation of Advanced Glycation End Products (AGEs), thereby delaying and preventing the onset of diabetic complications with minimal side effects. Free radicals have been shown to play a role in the formation of AGEs [Ref. B,C], just as they do in the development of a variety of diseases. Metformin is currently recommended as the primary anti-diabetic medication for overweight patients. Thiazolidinediones have been approved for use as monotherapy or in combination with a variety of other drugs [10-12]. Metformin, sulfonylureas, biguanides, and dipeptidyl peptidase 4 inhibitors are among the other medications. Several plant extracts have been studied in order to develop alternative diabetes treatment strategies [13-16]. This is done in an effort to reduce the severity of diabetic complications and the negative effects of current pharmacological agents. A variety of plants demonstrated hypoglycemic, hypotriglyceridemic, anti-lipid peroxidative, and anti-atherogenic properties in diabetic rats treated with streptozotocin (STZ) [17].

Flueggea virosa (Euphorbiaceae), also known as Chinese water berry, grows wild in many parts of the world, including tropical Africa, tropical Asia, Japan, the Middle East, and Australia. *F. virosa*'s various organs are used to treat a variety of illnesses, including arrhythmia, hepatitis, diabetes, HIV-related infections, fever, malaria, and epilepsy, among others. Other biological effects of the *Flueggea virosa* include antiplasmodial, trypanocidal, and antioxidant properties (ref C). *Flueggea virosa* has been found to contain a wide range of chemical components, including alkaloids, triterpenoids, resins, steroids, cardiac glycosides, bergenin, menisdaurin, and anthraquinones (ref D). Flavonoids, saponins, 11-O-acetyl bergenin, virosecuringine, ent-phyllanthidine, kaempferol, quercetin, gallic acid, daucosterol, and -sitosterol have also been discovered in *Flueggea virosa* (ref D). (ref. E). *F. virosa* and other *Flueggea* species can be used to treat malaria, jaundice, and a variety of other conditions [ref D]. An extract made from the leaves of the *Flueggea virosa* plant contains anti-diabetic properties due to the presence of flavinoid Rutin(ref D). It's possible that the extract promotes glucose uptake and metabolism while suppressing hepatic gluconeogenesis (ref E). The Anti-diabetic potential of *Flueggea virosa* needs to be further explored and studied extensively.

The goal of this study was to learn more about the anti-diabetic potential (preventing or delaying the development of diabetic complications) of a hydro-ethanolic extract of *Flueggea virosa* and to compare its efficacy to that of metformin, a well-known diabetes treatment.

Materials and methods

Chemicals and reagents

Streptozotocin (STZ) and all the reagents and chemicals used in the present work were of analytical grade.

Plant Extract preparation

Fresh leaves and twigs of *Flueggea virosa* were collected from Koyana forest, Maharashtra and identified by Botanical Survey of India, Western Regional Centre, Koregaon Road, Pune, Maharashtra, India. (Voucher No. BSI/WC/Tech/2015/309)

The fresh aerial parts of *Flueggea virosa* were washed under running tap water dried and ground to a fine powder. The powder (20 g) was extracted with distilled water and ethanol mixture (40:60%) in a Soxhlet apparatus for 8 h. The extracts were subjected to evaporation with Rota-evaporator (LABOROTA 4000WB Heidolph) and then oven-dried at 37°C. The extracts were stored at - 4°C until use.

Experimental animals

Male Wistar albino rats 8-12 weeks old weighing 190 to 200g were obtained from National Institute of Bioscience, Shirval, Pune, and the present study was carried out in animal house facility of Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune. A protocol was approved by the Institutional Animal Ethical Committee of Smt Kashibai Navale College of Pharmacy, Kondhwa, Pune, Maharashtra, India and as prescribed by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India was used in the present study. The rats were maintained in the laboratory under standard environmental conditions of temperature (25±2°C) and optimum humidity (40-60%), light and dark cycles of 12 h with 325 lux (light on at 8.00 & off at 20.00). The animals were kept individually in cages under clean and hygienic conditions. Autoclaved rice husk was spread at the bottom of each cage and replaced every morning. The rats were fed with unlimited supply of standardized pelleted diet and water. Only during fasting period the feed was taken out from the cages at night but water was available ad libitum.

Induction of diabetes

Diabetes was induced by a single intra-peritoneal (IP) injection of STZ (60 mg/kg) in freshly prepared citrate buffer (pH 4.5) to overnight (14 h) starved rats. They were subsequently given drinking water with sucrose (15 g/l) for 48 h to overcome the drug-induced hypoglycemia. This prevents immediate mortality as insulin is released by the damaged pancreas [20]. Five days later blood was collected from the tail to measure glucose using Glucometer [21]. All rats which had a blood glucose level exceeding 200 mg/dl were considered as being diabetic and were used for further studies.

Experimental protocol

Acute oral toxicity study of on healthy adult female Wistar albino rats was done as per OECD (Organization for Economic Cooperation and Development, 2006) guideline # 423. The rats were given a maximum dose of 2000 mg/kg body weight of *Flueggea virosa* extract. Dose progression was done in a sequence of 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg/kg per oral route. The rats were observed for mortality up to 14 days. Based on this pilot study of acute oral toxicity, the male rats were divided into five groups, each of six animals (n = 6). All the groups were treated as per following specifications.

Normal Control Group: Rats were non diabetic. These animals were only injected with the citrate buffer vehicle, on the same day the animals from other four groups were injected with STZ. Thereafter they were provided with the normal food and water.

Diabetic Control Group: The rats were injected with STZ (60 mg/kg, IP; single dose) and kept under observation for 48 h to check mortality. During this period they were provided with food and sucrose fortified drinking water (as prescribed above), thereafter they were given the normal food and water.

Metformin Group: The rats were first rendered diabetic with STZ as above. After 48 h they were force fed metformin (500 mg/kg) every morning for the next 21 days [22]. Metformin was used as a standard reference drug and as the positive control.

Plant Extract Group 1 The rats were first rendered diabetic with STZ as above. After 48 h they were fed 100 mg of hydro-ethanolic extract of *Flueggea virosa* /kg of body weight orally every morning for the next 21 days.

Plant Extract Group 2 The rats were first rendered diabetic with STZ as above. After 48 h they were fed 200 mg of hydro-ethanolic extract of *Flueggea virosa* /kg of body weight orally every morning for the next 21 days. Metformin and hydro-ethanolic extract of *Flueggea virosa* (100mg & 200 mg) were given orally once in a day for 21 days through a vehicle. Blood glucose was measured before treatment (0 day), and on 7th, 14th, and 21st day after treatment. Other biochemical parameters were measured after completion of the three weeks of treatment.

Experimental design

The body weight and fasting blood sugar of all animals were measured over the three weeks period of study. Blood glucose was measured using Glucometer (ACCU-CHEK Active Glucometer Monitor, Roche Diagnostic Australia Pty.Ltd.31 Victoria Avenue, Castle Hill, NSW 21, Australia.), by the glucose oxidase-peroxidase method [23]. All biochemical parameters were measured after the completion of the 21 days of treatment. Blood was collected by cardiac puncture after sacrificing the rats and was tested for effect of extract of *Flueggea virosa* on urea and blood urea nitrogen using the Berthelot method [24]. Creatinine was analyzed by Jaffe's colorimetric-kinetic method [25-26] and uric acid was measured by Urease-POD enzymatic colorimetric-kinetic method [27]. Total protein was measured by Biuret method [28-30]. After the completion of the treatment period all the rats were sacrificed by cervical dislocation, dissected and the pieces of kidney tissues were removed, weighed and fixed into 10% formalin solution. The tissues (4-5 mm³) were cut, dehydrated in alcohol gradation and embedded in paraffin wax (54°C to 58°C). Paraffin wax embedded tissue blocks were prepared and sectioned at 5µm on a microtome (Weswox Senior Rotary Microtom, Spencer 820 Type MT-1090A). The tissue sections were stained by haematoxylin and eosin [31] and stained slides were observed under Motic Digital Biological Microscope and photographed (Model No. BA 210 with colour corrected infinity optical system and imaging software).

Statistical analysis

The data obtained were expressed as mean ± SEM. Statistical analysis was performed on commercial software INSTAT 3.0, a demo version (Graph Pad Software, San Diego, CA). Unpaired t-test with two tail p value was used for statistical analysis. Significance level was at p< 0.05.

Results

1. Acute oral toxicity study

A hydro-ethanolic extract of *Flueggea virosa* was fed to the rats at maximum dose level of 2000 mg/kg. It was noted that this dose also did not induce mortality within 14 days. We also did not notice any adverse acute or chronic effects on the activity of the animals when tested in a locomotor activity instrument (INCO Make). During the experiment, the animals were healthy and active.

2. Effect of *Flueggea virosa* extract on body weight of Streptozotocin treated rats

Body weights of rats in the five groups were monitored during the 21 days of experimental period. As compared to control, significant decrease in body weight was observed on 7th (18.7%), 14th (23.55%) and 21st (30.64%) days in diabetic group. (Table1). Metformin treated animals showed 4.62% increase in body weight on day 21 as compared to initial weight and 34.50% significant increase compared to diabetic group. Administration of *Flueggea virosa* extract at the dose of 200 mg/kg resulted in a significant 30.17% increase in body weight on day 21 when compared to diabetic group and 6.16% increase in body weight than that of the initial weight of rats. When 200mg extract treated group was compared to metformin treated group then it was observed that extract (200mg/kg body weight) treated group (6.16%) showed slight increase in body weight of the rats on the day 21. Extract of *Flueggea virosa* at the dose 100 mg/kg showed less increase in the body weight (16.41% (P<0.001)) on day 21 when compare to diabetic group of the rats on day 21 and this group showed 9.76% reduction in body weight of rats when compared to the initial weight. The results have shown that *Flueggea virosa* extract at the dose 200 mg /kg and metformin significantly increase body weight in diabetic rats.

Table No. 1. Effect of *Flueggea virosa* extract on body weight of Streptozotocin treated rats.

Groups	Body weight (g)			
	0 Day	7 th Day	14 th Day	21 st Day
Control	204.23 + 4.25	212.81 + 3.97	217.82 + 2.72	223.24 + 3.52
Diabetic	191.71 + 6.56	173.05 + 5.28***	166.51 + 3.67***	154.82 + 3.24***
Metformin	196.02 + 3.31	192.83 + 9.48	243.00 + 14.37###	228.25 + 1.86###
Extract 200mg	189.80 + 4.65	186.24 + 7.26	192.04 + 6.72	201.51 + 4.91###
Extract 100mg	199.72 + 2.74	211.80 + 2.57##	183.30 + 6.22	180.23 + 4.88

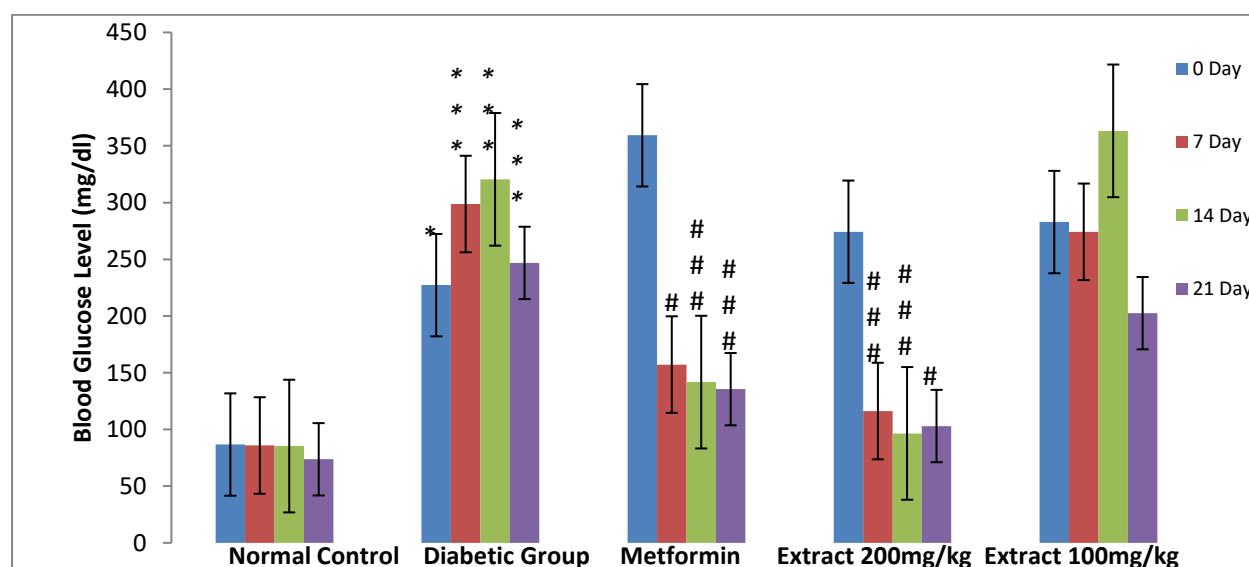
n = 6 rats/group. Values are mean + SEM. *** indicates (p<0.001) compared to control group. ## indicates (p<0.01), ### indicates (p<0.001) as compared to diabetic group.

As compared to control, significant (p<0.001) decrease in body weight was observed on 7th, 14th and 21st days in diabetic group. Metformin treated animals showed significant (p<0.001) increase in body weight as compared to diabetic animals. Extract of *Flueggea virosa* at the dose 200 mg/kg showed significant (p<0.001) increase in body weight on day 21 when compared to diabetic group. The apparent decrease in body weight of 100mg/kg *F. virosa* treated rats at 21 days was however not significant in comparison with the initial value (Table 2).

3. Effect of hydro-ethanolic extract of *Flueggea virosa* on blood glucose level

As compared to the control group, significant increase in blood glucose level was observed in diabetic animals on 7th day, 14th and 21st days (p<0.001) (Fig. 1). At the dose of 200mg/kg of *Flueggea virosa* after 21 days, there occurred significant decreased in blood glucose level on 7th, 14th days (p<0.001), and on 21st day (p<0.05) as compared to the diabetic rats. There was no significant reduction in blood glucose levels at a dose of 100mg/kg of *Flueggea virosa* compared to the diabetic group and 200mg/kg of *Flueggea virosa* (Fig. 1). Metformin treated group as expected showed decrease in blood glucose on 7th (p<0.05), 14th and 21st (p<0.001), days when compared with diabetic group. A dose of 100mg/kg of *Flueggea virosa* did not show any significant effect on blood glucose levels compared to the higher dose of extract of 200mg/kg.

Figure 1: Effect of hydro-ethanolic extract of *Flueggea virosa* on blood glucose level



Values are mean + SEM. * indicates (p<0.05), *** indicates (p<0.001) compared to control group. # indicates (p<0.05), ### indicates (p<0.001) as compared to diabetic group.

4. Effect of hydro-ethanolic extract of *Flueggea virosa* on urea, creatinine, uric acid, total protein and Blood Urea Nitrogen

Blood urea, creatinine, uric acid and blood urea nitrogen levels were elevated in the diabetic group as compared to control group by 82.19%, 163.77%, 100.43% and 151.59% respectively. The blood urea, creatinine, uric acid and blood urea nitrogen levels were significantly decreased (p<0.001) in Metformin treated animals by 32.11%, 52.20%, 45.14% and 47.86% respectively, when compared to diabetic group. The blood urea, creatinine, uric acid and blood urea nitrogen levels were significantly decreased (p<0.001) in 200mg/kg of *Flueggea virosa* extract treated animals by 34.26%, 42.86%, 46.44% and 49.18% respectively, as compared to diabetic group. The creatinine, uric acid and Blood urea nitrogen levels of rats treated with *Flueggea virosa* at dose of 100mg/kg also showed significant decrease (p<0.001) than that of diabetic rats by 27.47%, 47.52% and 37.80%.

Diabetic group showed significant decrease (p<0.001) in total serum protein by 31.94 % than the normal control rats, whereas rats treated with metformin and *Flueggea virosa* (dose of 200mg/kg and 100mg/kg) exhibited significant increase (p<0.001) in total protein level by 28.44 % and 42.42 %, 37.80% respectively when compared to the diabetic group.

Table 2. Effect of hydro-ethanolic extract of *Flueggea virosa* on several biochemical parameters in the blood of diabetic rats.

Groups	Blood Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)	Total Protein (gm/dL)	Blood urea nitrogen (mg/dL)
Control	43.18±1.39	0.69±0.16	2.31±0.20	6.20±0.04	16.94±0.53
Diabetic	78.67±2.29***	1.82±0.02***	4.63±0.09***	4.22±0.09***	42.62±0.33***
Metformin	53.41±2.21###	0.87±0.02###	2.54±0.30###	6.01±0.13###	22.22±0.43###
Extract 200mg	51.72±2.66###	1.04±0.02###	2.48±0.035###	5.42±0.08###	21.66±0.22###
Extract 100mg	66.42±2.21##	1.32±0.02###	2.43±0.15###	4.82±0.08##	26.51±0.36###

Values are mean + SEM. *** indicates (p<0.001) compared to normal control group. ## Indicates (p<0.01), ### Indicates (p<0.001) as compared to Diabetic group.

5) Effect of hydro-ethanolic extract of *Flueggea virosa* on histology of Kidney.

Section of kidney through cortex and medulla of control rats (Fig. 2A) did not show any pathological changes, except for a slight degree of cellular infiltration. Section of the kidney of diabetic rats (Fig. 2B) showed severe tubular swelling, dilatation of tubules, vascular changes, and moderate cellular infiltration, necrosis and tubular degeneration and casts formation. In the diabetic group severe glomerulopathy was also observed. In the diabetic group (Fig. 2B) kidney tissue showed proteinaceous casts in the tubular lumen of uriniferous tubules. Basement membrane of tubular cells were detached in some tubules, this showed tubular damage. The cellular infiltration, vascular changes were slightly lesser in rats treated with Metformin (Fig. 2C) as compared to other groups (Fig. 2 B, D, E). Moderate necrosis, tubular dilatation and degeneration were found in rats treated with 200mg/kg (Fig. 2 D) and 100 mg/kg (Fig. 2 E) *Flueggea virosa*. Mild to moderate tubular necrosis in the proximal tubule, was found in the sections of kidney of the Metformin, 200mg, and 100 mg extract treated rat groups (Fig. 2 C, D, E). In *Flueggea virosa* extract (200 and 100mg/kg body weight) treated (Fig. 2 D, E) diabetic kidney, tubular walls showed increase in the thickness and glomeruli and tubules were without proteinuria and hemorrhage. Moderate to mild glomerulopathy was found in Metformin, 200mg/kg and 100 mg/kg *Flueggea virosa* treated groups (Fig. 2 D, E).

6) Effect of extract of *Flueggea virosa* on histological changes in progression of diabetic nephropathy in diabetic rats.

Diabetic rats exhibited glomerular hypertrophy as significant decrease ($p < 0.001$) was seen in glomerular length, width and area when compared to normal control group. Diabetic group showed significant increase in ($p < 0.001$) thickening of glomerular membrane (GM), proliferation of mesangial cells and thickening and dilation of renal tubules (Table 3 A). Metformin and 200mg *Flueggea virosa* extract treated groups exhibited significant improvement in glomerular architecture and significant decrease ($p < 0.001$) in GM thickness, mesangial proliferation ($p < 0.01$) and tubular dilation ($p < 0.001$) when compared to diabetic group (Table 3 A).

Table 3. A. Summary of histological renal injury in normal, diabetic and Metformin and *Flueggea virosa* treated groups.

Group	Glomerulus Length μm	Glomerulus Width μm	Glomerulus Area μm^2	Membrane thickness μm	Mesangial proliferation μm	Tubular thickening μm	Tubular dilatation μm
Control	10.78 \pm 0.09	9.70 \pm 0.04	104.1 \pm 0.65	0.05 \pm 0.002	0.33 \pm 0.00	0.17 \pm 0.0	0.17 \pm 0.0
Diabetic	7.80 \pm 0.41***	4.94 \pm 0.22**	38.45 \pm 3.761**	0.16 \pm .012***	2.62 \pm 0.12***	2.21 \pm 0.28***	3 \pm 0.0***
Metformin	9.99 \pm 0.20###	6.52 \pm 0.40##	64.78 \pm 3.33###	0.082 \pm 0.002###	1.88 \pm 0.20#	1.33 \pm 0.09#	1.75 \pm 0.08###
200 mg Extract	10.63 \pm 0.06###	6.47 \pm 0.34##	67.3 \pm 3.24###	0.084 \pm 0.004###	1.66 \pm 0.092##	1.64 \pm 0.039	1.62 \pm 0.03###
100 mg Extract	8.46 \pm 0.45	4.70 \pm 0.29	40.03 \pm 4.36	0.092 \pm 0.004###	1.95 \pm 0.25#	1.79 \pm 0.25	1.82 \pm 0.25###

Values are mean + SEM. *** indicates ($p < 0.001$) compared to normal control group. # indicates ($p < 0.05$), ## indicates ($p < 0.01$), ### indicates ($p < 0.001$) as compare to Diabetic group.

Table 3. B. Summary of histological renal injury in normal, diabetic and Metformin and *Flueggea virosa* treated groups.

Group	Cast in lumen %	Necrosis and degenerative changes %	Collagen fiber deposition in interstitium %	Inflammatory cells /HPF %	Capillary thickening μm	congestion %	glycogen deposition %	Crescent formation %
Control	0.17 \pm 0.0	0.17 \pm 0.0	0.17 \pm 0.0	0.63 \pm 0.03	0.28 \pm 0.03	0.17 \pm 0.0	0.17 \pm 0.0	0.00 \pm 0.0
Diabetic	2.07 \pm 0.25***	2.93 \pm 0.07***	2.14 \pm 0.04***	13.74 \pm .73***	2.53 \pm .11***	2.72 \pm 0.03***	1.90 \pm 0.13***	2.71 \pm 0.10**
Metformin	1.13 \pm 0.20##	1.79 \pm 0.08###	1.38 \pm 0.25#	5.58 \pm 0.69###	1.29 \pm 0.15###	1.46 \pm 0.15###	1.33 \pm 0.12##	1.5 \pm 0.23###
200 mg Extract	1.5 \pm 0.054	1.95 \pm 0.03###	1.58 \pm 0.04	6.85 \pm 0.11###	1.58 \pm 0.11##	1.51 \pm 0.11###	1.633 \pm 0.034	1.72 \pm 0.060##
100 mg Extract	1.77 \pm 0.23	2.01 \pm 0.22###	1.80 \pm 0.26	7.26 \pm 0.47###	1.86 \pm 0.25#	1.90 \pm 0.22##	1.65 \pm 0.14	1.91 \pm 0.22#

Values are mean + SEM. *** indicates ($p < 0.001$) compared to normal control group. # indicates ($p < 0.05$), ## Indicates ($p < 0.01$), ### Indicates ($p < 0.001$) as compare to Diabetic group.

Diabetic rats developed the histological changes of early diabetic nephropathy, such as significant increase ($p < 0.001$) in collagen fiber deposition in interstitium, thickening of blood capillaries, glycogen deposition, crescent formation and congestion in kidney tissue in association with a progressive increase in kidney leukocytes or inflammatory cells (Table 3 B). Diabetic group exhibited elevated cast deposition in lumen and significantly high percentage of necrosis or degenerative changes in renal tissues when compared to normal control group.

Metformin treated group showed significant reduction ($p < 0.001$) in cast deposition in lumen, collagen fiber deposition, thickening of blood capillaries, number of inflammatory cells, glycogen deposition, crescent formation and congestion in kidney tissue, this group also showed significant decrease ($p < 0.001$) in necrosis or degenerative changes in renal tissues (Table 3 B).

The 200mg and 100mg *Flueggea virosa* extract treated groups showed significant decrease ($p < 0.001$) in number of inflammatory cells, congestion and necrosis of renal tissues.

Discussions

The practice of using of medicinal plants to cure various diseases has been a reliable source of medication in several Asian countries [3]. Though ancient, the practice is still acknowledged and widely used in various countries under various names such as Ayurveda, Unani etc. Extensive research in this field has shown that particular extracts of the medicinal plants have specific effects on digestive, circulatory, respiratory systems [32]. In developed and developing countries, the focus in the field of herbal medicines has gained popularity because of ease of availability and perhaps minor side effects. This has led to the use of alternative medicines naturally available for treatment of diseases such as diabetes.

During the experimental period of 21 days, the control rats appeared healthy, active and gained body weight. Diabetic group showed mortality rate of about 50%. Body weight loss in diabetic group was due to reduction in intake of food [33-35] and by degradation of body proteins and muscle wasting caused by diabetes [36]. This finding is also corroborated by the present study showing a significant decrease of the total blood protein in the diabetic rats (Table 2). Metformin and 100 mg/kg of *Flueggea virosa* extract treated groups showed 33% mortality rate which was less than diabetic rats. The 200mg/kg of *Flueggea virosa* treated group showed low mortality rate (25%) than that of any other group.

Our studies revealed that the rats rendered diabetic by streptozotocin underwent progressive weight loss (Table 1. $p < 0.001$) as compared to the control animals which gained body weight. This is a common finding for all diabetic animals as shown for example by Zafar Naqvi [37]. On the other hand Metformin and 200mg/kg extract of *Flueggea virosa* treated animals showed significant increase in body weight, probably due to improved glucose metabolism.

The hypoglycemic activity of plants is due to the presence of compounds which has insulin-like substances [38]. Some plants may stimulate B cells to produce more insulin [39]. Plants have high amounts of fibers which delay carbohydrate absorption [40] while some plants have a regenerative effect on pancreatic tissue [41-44]. The oral treatment of 200mg/kg of *Flueggea virosa* and Metformin once daily resulted in significant reduction in blood glucose level in STZ-induced diabetic rats. Insulin promoting activity of *Flueggea virosa* is similar to Metformin [45]. Similar activity has also been observed earlier by Rahuja et. al [46].

The findings of the present experiment suggest that Metformin and extract of *Flueggea virosa* decrease blood glucose by increasing the pancreatic secretion of insulin from the remnants of β cells of the pancreas or its responsiveness. [47]. Based on the results of the present study it can be concluded that hydro-ethanolic extract of *Flueggea virosa* has anti-diabetic, anti-hyperglycemic properties. The extract of aerial parts of *Flueggea virosa* caused a significant decrease in blood glucose level and the extract has potential as hypoglycemic agent equally effective as compared to standard drug Metformin.

Diabetic nephropathy is one of the most serious microvascular complications of diabetes mellitus. In present study, STZ induced diabetic rats showed significant high levels of blood urea, serum creatinine, blood urea nitrogen, uric acid and low levels of total protein. The increased protein breakdown and renal dysfunction might be the reason for high levels of urea and creatinine in the serum [48]. Diabetic rats treated with a hydro-ethanolic extract of *Flueggea virosa* (100mg/kg and 200mg/kg) showed significant reduction in the levels of blood urea, serum creatinine, blood urea nitrogen and uric acid when compared to diabetic group (Table 2). This indicated that the extract of *Flueggea virosa* significantly prevented the development of renal damage in diabetic rats.

Urea is the major metabolic product of protein metabolism and blood urea nitrogen acts as a significant marker of renal dysfunction [49]. Blood urea nitrogen has declined significantly in the *Flueggea virosa* and Metformin treated rats.

Previously several studies have shown that serum protein levels decrease in diabetic rats [50, 51] and protein metabolism is also changed, it reduces the utilization of amino acids by tissues, increases proteolysis and decreases protein synthesis, it increases urea production in liver [52]. Protein glycosylation is associated with excess urea and glucose in blood. Renal vascular changes severely damage kidney; this condition causes excretion of protein in the urine [53].

In STZ-induced diabetic rats reduction in serum total protein was observed in the present study. It may be due to decreased amino acid uptake or decreased concentration of essential amino acids or excess glycogenic amino acids get converted into carbon dioxide and water or due to reduction in protein synthesis due to unavailability of sufficient mRNA or due to combined effect of all these factors [54]. On the other hand, in the *Flueggea virosa* extract treated group and standard Metformin treated diabetic rats the total protein levels in the blood were close to the normal control (Table 2). *Flueggea virosa* and Metformin treated groups showed decreased levels of blood urea, uric acid, blood urea nitrogen and creatinine as compared to diabetic rats. In the *Flueggea virosa* treated groups and standard Metformin treated diabetic rats the protein levels were near to normal range (Table 2).

Glomerular hypertrophy, thickening of the glomerular basement membrane, proliferation of the glomerular mesangial cells and obstruction in glomerular capillaries may develop end stage renal disease in diabetes mellitus [55, 56]. In the current studies, kidney histo-pathological results exhibited dilatation of proximal and distal tubules in the cortex (Figure 2 B), it was associated with the primary effect of diabetic state of the kidney. The secondary effect or the individual response factor was associated with inflammatory processes [57].

Diuresis is a common pathological condition associated with diabetes which was caused due to structural changes in glomerulus [58]. Metformin and 200mg *Flueggea virosa* extract treated rats exhibited improvement in glomerular architecture and significant reduction in Glomerular thickness, mesangial proliferation, collagen fiber deposition, thickening of blood capillaries, number of inflammatory cells, glycogen deposition, crescent formation, tubular dilation and congestion in kidney tissue. Metformin and 200mg *Flueggea virosa* extract treated animals showed significant decrease in necrosis or degenerative changes in renal tissues (Table 3 A & B).

Flueggea virosa extract caused showed reversal of changes in the diabetic state at the cellular level. It indicates anti-diabetic potential of the extract. The clinical pathology of diabetic nephropathy is strongly related to the morphological changes, which are associated with the mesangial expansion in kidney section of diabetic rats [59, 60]. The mesangial expansion is a serious destructive structural change leading to loss of renal function in diabetes. The mesangial proliferation increase glomerular volume which decreases the filtration surface of glomerulus. Due to such glomerulopathy, animals developed renal end stage disease [44, 61].

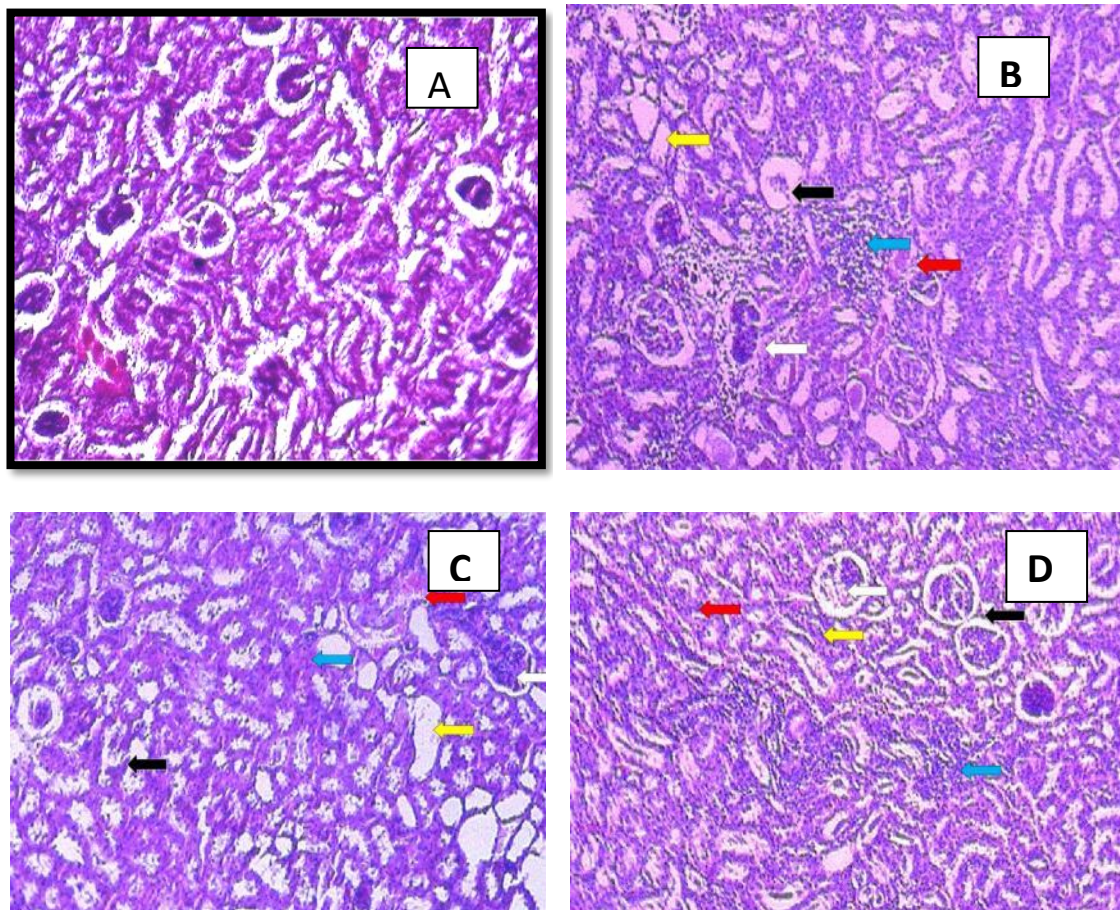
In present study, kidney sections of STZ induced diabetic rats treated with Metformin and 200 mg/kg *Flueggea virosa* extract have showed improvements indicating moderate to mild glomerulopathy, reduction in tubular swelling and dilation. Thijs W. Cohen Tervaert et al. a classified diabetic nephropathy, as class I and class II type [62]. The diabetic rats had both glomerular basement membrane thickening (class- I DN) and the mild mesangial expansion (class II- DN) and mild mesangial expansion (class II-DN) classes of diabetic nephropathy. Metformin and 200mg *Flueggea virosa* extract treated groups showed mild glomerular basement membrane thickening and mesangial expansion as compared to the diabetic rats. It appears that the destructive morphological changes partially reversed to normal in the kidney of the treated animals.

Conclusion

It may be concluded from the study that (a) the hydro-ethanolic extract of *Flueggea virosa* has significant hypoglycemic effect, moderate renoprotective and delaying effect on progression of diabetic nephropathy, (b) similar results were obtained when the STZ-induced diabetic rats were treated with 200mg/kg/day of plant extract for 21 days. The results of this study suggest that Metformin and *Flueggea virosa* extract lower blood glucose by increasing the pancreatic secretion of insulin from the remnants of β cells of the pancreas or its responsiveness [47]. This resulted in low mortality and a significant increase in body weight, most likely as a result of improved glucose metabolism. *Flueggea virosa* extract significantly reduced the development of renal damage in diabetic rats. Treated rats exhibited improvement in glomerular architecture and decrease in necrosis or degenerative changes in renal tissues. *Flueggea virosa* extract reversed changes in the diabetic state at the cellular level. The destructive morphological changes appear to have been partially reversed in the kidneys of the treated animals [58-60].

In conclusion our study demonstrates that the hydro-ethanolic extract of *Flueggea virosa* thus reduces hyperglycemia significantly and diabetic nephropathy moderately.

Figure 2: A- Normal Control, B- Diabetic, C- Metformin, D- Extract 200mg/kg, E- Extract 100mg/kg



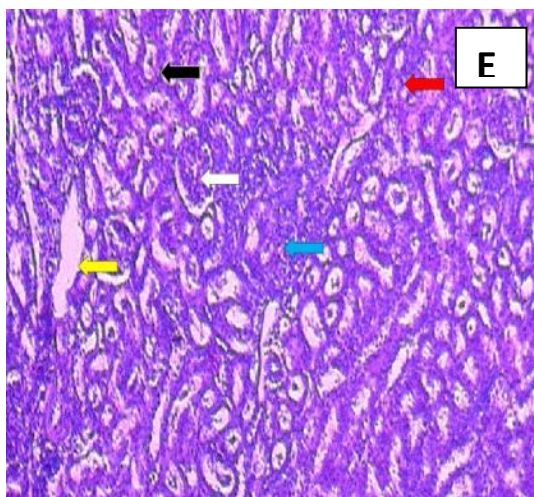


Figure 2: A- Normal Control, B- Diabetic, C- Metformin, D- Extract 200mg/kg, E- Extract 100mg/kg

Photograph showing, tubular swelling and dilatation of tubules (yellow arrow), vascular changes (red arrow), cellular infiltration (blue arrow), necrosis and tubular degeneration and casts formation (black arrow), glomerulopathy (white arrow) H& E stain 100X

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Disclosure

This is an original research manuscript performed by authors without any external funding. The research findings has not been published or submitted in this manuscript form elsewhere. All the authors have participated in experimental design, research work, collection and data processing and writing the manuscript. The authors declare that they have no conflicting interests.

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