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Original Article

Antidiabetic and hepato-renal protective effects of medicinal plants in STZ induced diabetic rats

Efeitos antidiabéticos e hepatorrenais de plantas medicinais em ratos diabéticos induzidos por STZ

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Abstract

The antidiabetic and hepato-renal protective effects of *Citrullus colocynthis* and *Momordica charantia ethanol* extracts were investigated in streptozotocin (STZ) induced diabetic male albino rats. Diabetic rats were treated with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* mixed extract at a dose of 250 mg /kg body weight per oral per day for 21 days. The mean body weight of all the diabetic rat groups on day 1 of treatment (day 10 of diabetes) was significantly lower than the normal control rat group (P<0.05). The blood glucose level of all the diabetic rat groups on day 1 of treatment (day 10 of diabetes) was significantly (P<0.05) higher (> 200 mg/dl) than the normal control rat group (95.5 ± 2.7). At the end of treatment (day 21), the diabetic rats treated with plant extracts showed significant increase (P<0.05) in body weight and significant (P<0.05) reduction in blood glucose level when compared to diabetic control animals. Significant increase (< 0.05) was observed in the serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea and creatinine levels of diabetic control rat group. The serum levels of these liver and kidney-related parameters of diabetic rats treated with plant extract were significantly lower when compared to diabetic control rat group (p < 0.05). Photomicrographs of liver and kidney microsections from diabetic rats treated with these plant extracts showed amelioration in the hepato-renal histoarchitectures. It was concluded that the *C. colocynthis* and *M. charantia* methanol extracts are antidiabetic and hepato-renal protective in STZ induced diabetic male rats. Treatment of the diabetic rats with *C. colocynthis* + *M. charantia* mixed extract is more effective in the amelioration of diabetes and hepato-renal injuries in STZ induced diabetic male rats.

Keywords: *Citrullus colocynthis*, *Momordica charantia*, extracts, liver, kidneys.

Resumo

Os efeitos protetores antidiabéticos e hepatorrenais dos extratos etanólicos de *Citrullus colocynthis* e *Momordica charantia* foram investigados em ratos albinos machos diabéticos induzidos por estreptozotocina (STZ). Ratos diabéticos foram tratados com extrato misto de *C. colocynthis*, *M. charantia* ou *C. colocynthis* + *M. charantia* na dose de 250 mg/kg de peso corporal por via oral por dia durante 21 dias. O peso corporal médio de todos os grupos de ratos diabéticos no dia 1 de tratamento (dia 10 de diabetes) foi significativamente menor do que o grupo de ratos controle normal (P < 0,05). O nível de glicose no sangue de todos os grupos de ratos diabéticos no dia 1 de tratamento (dia 10 de diabetes) foi significativamente (P < 0,05) maior (> 200 mg/dl) do que o grupo de ratos controle normal (95,5 ± 2,7). Ao final do tratamento (dia 21), os ratos diabéticos tratados com extratos vegetais apresentaram aumento significativo (P < 0,05) no peso corporal e redução significativa (P < 0,05) na glicemia quando comparados aos animais controle diabéticos. Aumento significativo (< 0,05) foi observado nos níveis séricos de bilirrubina, alanina transaminase (ALT), aspartato transaminase (AST), fosfatase alcalina (ALP), ureia e creatinina do grupo controle diabético. Os níveis séricos desses parâmetros hepáticos e renais de ratos diabéticos tratados com extrato vegetal foram significativamente menores quando comparados ao grupo controle de ratos diabéticos (p < 0,05). Fotomicrografias de microseções de fígado e rim de ratos diabéticos tratados com esses extratos vegetais mostraram melhora nas histoarquiteturas hepatorrenais. Concluiu-se que os extratos metanólicos de *C. colocynthis* e *M. charantia* são antidiabéticos e hepatorrenais protetores em ratos machos diabéticos induzidos por STZ. O tratamento de ratos diabéticos com extrato misto de *C. colocynthis* + *M. charantia* é mais eficaz na melhora do diabetes e lesões hepatorrenais em ratos machos diabéticos induzidos por STZ.

Palavras-chave: *Citrullus colocynthis*, *Momordica charantia*, extratos, fígado, rins.

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1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. This condition is resulting from the lack of insulin-secretion from the beta cells of the pancreas and desensitization of insulin receptors. There two types of diabetes, viz type 1 and type 2. Type 1 diabetes is also called insulin-dependent diabetes mellitus which develops due to loss of insulin secreting beta cells of pancreatic Langerhans islets. Type 2 diabetes is also called non-insulin-dependent diabetes mellitus which is due to a reduction in sensitivity to insulin or insulin resistance. Major symptoms of diabetes include polydipsia, polyuria, polyphagia, pruritus, peripheral neuropathy, weakness, fatigue (Marks et al., 1996). Increased glucose level disturbs the normal plasma membrane lipid content by initiating peroxidation. This results in the production of reactive oxygen species (Wright Junior et al., 2006). The liver and kidney are more susceptible to this oxidative stress (Sulaiman, 2019). A chronic form of diabetes disturbs normal liver functioning and produces diseases like fatty liver, inflammation, etc. A chronic form of diabetes may also cause diabetic kidney disease (DKD) or diabetic nephropathy (Alicic et al., 2017).

According to the World Health Organization 2016 report (WHO, 2016), about 80% of diabetic people are using medicinal plants for the treatment. Medicinal plants contain active phytochemicals *i.e* Polyphenols, terpenoids, charentin, memordian, crypoxanthin these chemicals regulate metabolic activities (Ebrahimi et al., 2016). *Citrullus colocynthis* commonly known as "bitter apple" is found in Europe, Asia, and Pakistan. *C. colocynthis* is recognized to have antioxidant, antidiabetic, hypolipidemic, anti-cancer, hepato-protective, and anti-inflammatory assets (Alhawiti, 2018). *Momordica charantia* (bitter gourd or bitter melon) can alter the metabolism of intracellular biomolecules for the alleviation of Type 2 DM symptoms (Alam et al., 2015). *Momordica charantia* has numerous active ingredients such as charantin, insulin-like peptide, glycosides, momordicin, and oleanolic acids (Sathishsekar and Subramanian, 2005). The objective of the present study was to explore the antidiabetic, hepatoprotective and nephroprotective effects of *C. colocynthis* and *M. charantia* ethanol extracts in diabetic Albino male rats.

2. Materials and Methods

2.1. Collection of plant materials and extraction

The *C. colocynthis* and *M. charantia* dry fruits were obtained from a trader of medicinal plants in local market at Lahore. Both plant fruits were authenticated by the taxonomists in the Department of Botany University of Lahore. The fruits of both plants were rinsed with distilled water and then shade dried. The dried fruits were powdered separately by a mechanical grinder. The powder form of each plant fruit in amount of 200 g was separately soaked in 500 mL of 70% ethanol in 1000 mL glass beaker. After 3 days of soaking and occasional shaking, each soaked material was filtered through Whatman no.42 filter paper. The soaking and filtration process for each plant fruit powder was repeated twice. Each filtrate was concentrated by a rotary evaporator. Finally, the extract of each plant powder was obtained in paste form of brown colour. The prepared extracts were stored in glass vials at 4^{0}C in refrigerator till further use.

2.2. Animal used

For experiment, 30 male albino rats, weighing 150-300 g were bought from the Animal Unit, The University of Lahore, Lahore. The animals were placed in rodent cages and provided food and water, *ad libitium*. The care and the use of the animals for experiment were according to the guidelines of institutional animal ethical committee of the University of Lahore.

2.3. Acute toxicity test

Four doses, each of the *C. colocynthis* and *M. charantia* extract were orally administered to four male albino rat groups (four rats in each group) according to the OECD guidelines no. 420 for determination of acute toxicity. The four doses were 100, 200, 400, and 800 mg per kilogram body weight per oral (mg/kg b. w. /o). In rats, the symptoms of toxicity and mortality were observed. All the doses tested were found safe.

2.4. Induction of diabetes

Thirty male albino rats were used in the experiment. Out of the 30 rats, 24 rats were used for induction of diabetes. Streptozotocin is commonly used for experimental induction of diabetes in animal models (Wu and Yan, 2015). For induction of diabetes type 1, to each rat, STZ was injected intraperitoneally at a dose of 75 mg /kg b. w. dissolved in 1.5 mL normal saline (0.9% w/v) in the over-night fasting male rats. After 12 hours of STZ injection, each rat was orally given 2 mL of 5% glucose solution through esophageal catheter to prevent the rats from STZ induced hypoglycemic shock. Blood glucose level was regularly checked by using glucometer by tail tipping method. The rats with 200 mg/dl of blood glucose levels were considered diabetic and used for the experiment.

2.5. Experimental design

After 10 days of induction of diabetes in rats, the proper experiment was started. Thirty rats were divided into 5 groups with 6 rats in each group (n=6) and treated with the relevant extract of plants by using oral gavage for 21 days. The dose of each extract used was 250 mg/kg body weight which was selected from the range of doses tested in acute toxicity test. The following is the grouping detail:

Group 1: This group was a normal control group of male albino rats, orally treated with 1 mL normal saline for 21 days.

Group 2: This group was diabetic control group of male albino rats orally treated with 1 mL normal saline (1 mL /kg b. w. /o./day) for 21 days.

Group 3: This was a group of diabetic rats that were orally treated with *C. colocynthis* methanol extract (250 mg /kg b. w. /o./day) in 1 ml normal saline for 21 days.

Group 4: This was a group of diabetic rats that were orally treated with *M. charantia* methanol extract (250 mg /kg b. w. /o./day) in 1 ml normal saline for 21 days.
Group 5: This was a group of diabetic rats that were orally treated with mix extracts of *C. colocynthis* and *M. charantia* (125+125 mg /kg b. w. /o./day) in 1 mL normal saline for 21 days.

2.6. Determination of body weight and blood glucose level

The body weight of each rat of each group was recorded on day 1 of the experiment (day 10 of diabetes) and at the end (day 21) of the experiment by tail tipping method. Similarly, blood glucose level was recorded on day 1 of the experiment (day 10 of diabetes) and at the end (day 21) of the experiment by using a glucometer by tail tipping method.

2.7. Blood collection and serum isolation

At the end of the experiment, the overnight fasting rats were sequentially anesthetized with inhaled chloroform. The animals were sequentially restricted on dissected board and then dissected. 2.5 mL blood was collected from the hear chamber of each rat through 3.5 mL syringe with 19-gauge needle and then expelled into coagulant coated glass tube and labeled accordingly. A blood specimen was collected for the biochemical analysis. Blood samples were then centrifuged for 5 minutes at 5000 rpm through centrifuge (Shimazu, Japan). The serum samples were stored in properly labeled eppendorf tubes which were then used for biochemical tests.

2.8. Study of liver and kidney

The levels of liver-related parameters such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin in serum were estimated through COBAS chemistry automation machine using Roche Diagnostic kits. In addition, liver was removed from each dissected animal and rinsed in normal saline and then sections were taken. The sections were fixed in 10% formalin solution, dehydrated with 100% ethanol solution and embedded in paraffin. The sections were then processed into 4 - 5 µm thick sections and stained with hematoxylin-eosin (HE) and observed under a biological microscope and images were taken with attached camera.

The levels of kidney related parameters such as urea and creatinine in serum were estimated through COBAS chemistry automation machine using Roche Diagnostic kits. In addition, sections were taken from the kidney of each rabbit for histopathological study. The procedure of kidney sections processing was the same as describe for the liver. Photomicrographs of kidney micro-sections were taken with the help of camera attached with microscope.

2.9. Statistical analysis

Results were presented as means + standard error of means (SEM). The means were compared by using Tukey's Test of Post Hoc Multiple comparison in One Way Anova. For these analysis computers software SPSS 16.0 was used.

3. Results

During the present experimental study, no rat died in any rat group. The rats in group 1 (normal control group) appeared healthy and were gaining weight and active. The rats in the rest of the groups were appearing ill. However, the rats in group 3, 4 and 5 which were treated with plant extracts showed recovery from ill appearance with time.

3.1. Body weight and blood glucose level

The mean body weight and blood glucose levels of all the rat groups were noted on day 1 of treatment (day 10 of diabetes) (Table 1). The mean body weight of all the diabetic rat groups (group 2, 3,4 and 5) were significantly lower than the normal control rat group ($P<0.05$). Similarly, the blood glucose level of all the diabetic rat groups (group 2, 3,4 and 5) was significantly ($P<0.05$) higher (> 200 mg/dl) than the normal control rat group (95.5 ± 2.7).

The mean body weight and blood glucose levels of all the rat groups were also noted at the end of treatment (day 21) (Table 2). The mean body weight of diabetic control rat group was 189.6 ± 2.7 gram which was significantly lowest when compared with the normal control or any of the remaining group (> 200 gram) ($P<0.05$). The mean body weight of diabetic rat groups treated with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* combined extract were significantly higher when compared with the diabetic control rat group ($P<0.05$). The mean body weight of rat group treated with the mixed extracts was significantly higher when compared with the rat groups treated with *C. colocynthis* or *M. charantia* extract ($P<0.05$). Similarly, the mean blood glucose level of diabetic control rat group was 211.6 ± 2.1 mg/dl which was significantly

Table 1. The body weight and blood glucose levels of normal control and diabetic rat groups on day first (day 10 of diabetes induction) of treatment with plant extracts.

Animal Groups	Body weight (g)	Blood glucose level (mg/dl)
1 Normal control	230.5 ± 6.2^b	95.5 ± 2.7^a
2 (Diabetic control)	185.4 ± 3.3^a	219.4 ± 2.4^b
3 (***C. colocynthis* extract**)	181.3 ± 5.5^a	213.2 ± 3.5^b
4 (***M. charantia* extract**)	179.6 ± 4.2^a	215.6 ± 2.2^b
5 (***C. colocynthis* +*M. charantia* combined extracts**)	178.3 ± 3.4^a	220.3 ± 3.4^b

Means sharing no superscript letter are significantly different at $P< 0.05$.

higher when compared with the normal control or any of the remaining groups (P<0.05). The mean blood glucose level of diabetic rat groups treated with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* combined extract was significantly lower (< 140 mg/dl) when compared with the diabetic control rat group (P<0.05). The mean blood glucose level of rat group treated with the mixed extracts was significantly lower when compared with the rat groups treated only with mixed extracts of *C. colocynthis* or *M. charantia* (P<0.05).

3.2. Effect on liver

3.2.1. Liver-relatedbiochemical parameters

The level of liver-relatedparameters such as albumen, bilirubin, ALT, AST and ALP of normal control, diabetic control and extract treated rabbit groups were estimated at the end (day 21) of treatment (Table 3). A significant increase ($p < 0.05$) was observed in the levels of these biochemical parameters in the diabetic control and extract treated rabbit groups when compared to the normal control group. However, a significant ($p < 0.05$) decrease was observed in all the extract treated groups when compared to the diabetic control group.

3.2.2. Liver histopathology

During the histopathological study of the liver, the photomicrograph of liver from control group (Figure 1a) showed normal hepatic architecture. Photomicrograph of liver from diabetic control group (Figure 1b) showed mild hydropic degeneration of hepatocytes. There was focal presence of aggregates of lymphocytes in portal tracts. Photomicrograph of liver from group treated with extracts of *C. colocynthis* (Figure 1c) and *M. charantia* (Figure 1d) showed some improvement in hepatic architecture. Photomicrograph of liver from group treated with mixed extracts of *M. charantia* and *C. colocynthis* (Figure 1e) showed more reccovery towards normal hepatic architecture.

3.3. Effect on kidney

3.3.1. Kidney related biochemical parameters

The level of kidney related parameters such as serum levels of urea and creatinine of normal control, diabetic control and extract treated rabbit groups were estimated at the end (day 21) of treatment (Table 4). A significant increase ($p < 0.05$) in the levels of these biochemical parameters was observed in the diabetic control rat group when compared to normal control. However, a significant ($p < 0.05$) decrease in the level of these parameters was observed in the diabetic rat groups treated with plant extracts compared to diabetic control group.

3.3.2. Kidney histopathology

During the histopathological study of the kidney, photomicrograph of kidney from control group (Figure 2a) showed normal renal capsule, nephrons and tubules. Photomicrographs of kidney from diabetic control group (Figure 2b) showed mild to moderate renal tubular degeneration. There were found areas with mononuclear cells infiltration in intratubular areas. Photomicrographs of the kidneys from rabbit groups treated with *C. colocynthis* extract (Figure 2c) or *M. charantia* extract (Figure 2d) showed some improvement in the architecture

Table 2. The body weight and blood glucose levels of normal control and diabetic rat groups at the end (day 21) of treatment with plant extracts.

Animal Groups	Body weight (g)	Blood glucose level (mg/dl)
1 Normal control	281.2 ± 3.7	101.2 ± 3.2[a]
2 (Diabetic control)	189.6 ± 2.7	211.6 ± 2.1[d]
3 (***C. colocynthis* extract**)	211.6 ± 2.4	165.5 ± 5.4[c]
4 (***M. charantia* extract**)	213.2 ± 3.1	159.4 ± 5.3[c]
5 (***C. colocynthis* +*M. charantia* combined extracts**)	228.2 ± 4.6	138.3 ± 7.2[b]

Means sharing no superscript letter are significantly different at P< 0.05.

Table 3. The mean serum levels of liver-related biochemical parameters of normal control and diabetic rat groups at the end (day 21) of treatment with plant extracts.

Parameters	Feeding groups				
	Normal control	Diabetic control	*C. colocynthis*	*M. charantia*	*C. colocynthis* +*M. charantia*
Bilirubin	0.02±0.01[b]	0.08±0.01[a]	0.04±0.01[a]	0.06±0.01[ab]	0.03±0.01[b]
ALT (U/l)	39.3 ±3.3[a]	62.7±2.2[d]	53.5±2.4[bc]	57.4±3.6[c]	45.7±3.1[b]
AST (U/l)	131±1.3[a]	203±1.3[e]	185±1.3[d]	173±1.3[bc]	166±1.3[b]
ALP (U/l)	123±1.3[a]	212±1.3[e]	185±1.3[d]	172±1.3[bc]	166±1.3[b]

Means sharing no superscript letter are significantly different at P< 0.05.

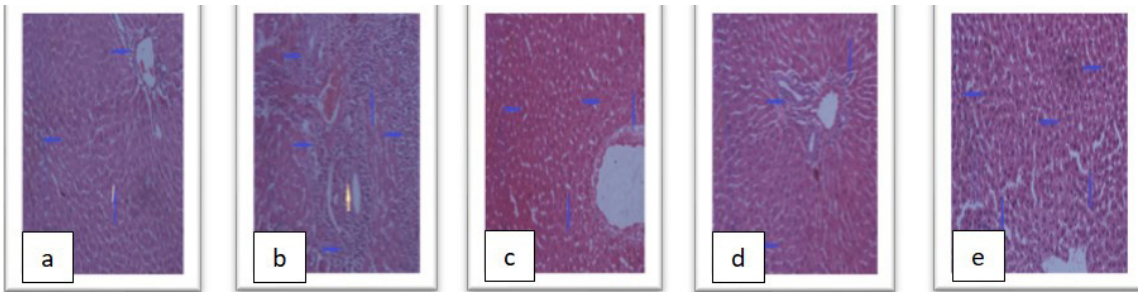

Figure 1. Photomicrographs of liver micro-sections of different rat groups. a: normal control rat group; b: diabetic control rat group; c: diabetic rat group treated with *C. colocynthis*; d: diabetic rat group treated with *M. charantia*; e: diabetic rat group treated with combination of *C. colocynthis* and *M. charantia* extracts.

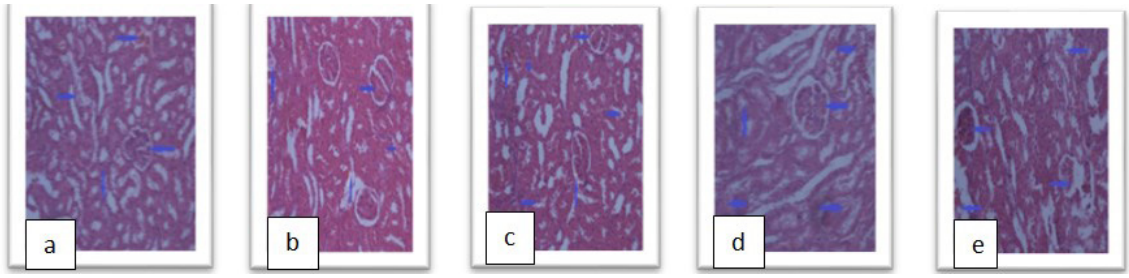

Figure 2. Photomicrographs of kidney micro-sections of different rat groups. a: normal control rat group; b: diabetic control rat group; c: diabetic rat group treated with *C. colocynthis*; d: diabetic rat group treated with *M. charantia*; e: diabetic rat group treated with combination of *C. colocynthis* and *M. charantia* extracts.

Table 4. The mean serum levels of kidney related biochemical parameters of normal control and diabetic rat groups at the end (day 21) of treatment with plant extracts.

Parameters	Feeding groups				
	Normal control	Diabetic control	*C. colocynthis*	*M. charantia*	*C. colocynthis* +*M. charantia*
Urea (mg/dL)	0.02±0.01^{b}	0.08±0.01^{a}	0.04±0.01^{a}	0.06±0.01^{ab}	0.03±0.01^{b}
Creatinine (mg/dL)	0.35 ± 0.02^{a}	0.8±0.04^{d}	0.6±0.03^{bc}	0.4±0.06^{b}	0.4±0.04^{b}

Means sharing no superscript letter are significantly different at P< 0.05.

of renal capsule, nephrons and tubules. There was little mononuclear cells infiltration and inflammation in intratubular areas. Photomicrograph of kidneys from rabbit groups (E) treated with mixed extracts of *M. charantia* and *C. colocynthis* (Figure 2e) also showed improvement in renal histoarchitecture.

4. Discussion

Diabetic is a condition of hyperglycemic state with general symptoms including weight loss, increased trust, hunger, and complications like diabetic nephropathy, retinopathy and fatty liver disease. Diabetic complications are connected to oxidative stress which occurred due to the formation of oxidants (Nasr et al., 2016). In the present study, diabetes was induced in male albino rats through intraperitoneal injection of STZ at a dose 75 mg /kg b. w. Streptozotocin (STZ) is a chemical widely used for induction of diabetes in animal models (Lenzen, 2008). In rodents' diabetes can be induced by a single STZ injection (Yin et al., 2006). Streptozotocin, a broad-spectrum antibiotic (Vavra et al., 1959) contains deoxy form of glucose molecule which is linked with methylnitrosourea moiety and directs the chemical to the β cells of pancreatic Langerhans's islets (Johansson and Tjalve, 1978). This results in the damage of pancreatic Langerhans's islets. Streptozotocin especially recognizes the glucose transporter 2 receptor which is abundant on β cells plasma membrane (Lenzen, 2008). Traditional medicinal plants are used throughout the world for the treatment of diabetes (Elalfy et al., 2019). The study of such medicines might offer a natural key to find new antidiabetic drugs (Marles and Farnsworth, 1995). In the present study, the protective effects of oral administration of *M. charantia* and *C. colocynthis* methanol extracts were studied in STZ-induced diabetic male albino rats. The parameters studied during this research were body weight, blood

glucose level, liver-relatedserum biochemical markers i.e., bilirubin, ALT, AST, and ALP and Kidney related serum biochemical markers i.e., urea and creatinine. This study also involved the histopathological study of liver and kidney of diabetic rats.

During the present study, the mean body weight and blood glucose levels of all the rat groups were noted on day 1 of experiment (day 10 of diabetes) (Table 1). The mean body weight of all the diabetic rat groups (group 2, 3,4 and 5) was significantly lower than the normal control rat group ($P<0.05$). The reduction in the body weight of diabetic animals is similar with the previous report (Oyedemi et al., 2011). This decrease in body weight in STZ induced diabetic animals is due to degradation of structural proteins and muscle wasting (Oyedemi et al., 2011). The blood glucose levels of all the diabetic rat groups (group 2, 3,4 and 5) were significantly ($P<0.05$) higher (> 200 mg/dl) than the normal control rat group (95.5 ± 2.7). During the study blood glucose level on day 1 (day 10 of diabetes), the blood glucose levels of all the diabetic rat groups (group 2, 3,4 and 5) were significantly ($P<0.05$) higher (> 200 mg/dl) than the normal control rat group (95.5 ± 2.7). Streptozotocin administration causes the suppression of insulin and consequently abnormally elevated blood glucose level (Nastaran, 2011). The type of diabetes induced during this study was type 1 as it was induced by injection of a single dose of STZ. It is already reported that injection of single dose of STZ causes Type 1 diabetes in rodents (Yin et al., 2006). Type 2 diabetes can be induced by STZ injection after administration of nicotinamide (Szkudelski, 2012). At the end of experiment (day 21 of treatment with plant extracts), the diabetic rats treated with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* mixed extract gained weight (Table 2). Their body weight were significantly higher when compared with the diabetic control rat group ($P<0.05$). Similarly, a decrease in the mean blood glucose level of diabetic rat groups treated with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* mixed extract was observed. Their blood glucose level was significantly lower (< 140 mg/dl) when compared with the untreated diabetic control rat group ($P<0.05$). The treatment of diabetic rats with mixed extract was found more effective in decreasing blood glucose level. This improvement in body weight and decrease in the blood glucose level of diabetic rats treated with plant extracts indicate the hypoglycemic effect of plant extracts. This antidiabetic effect of these plant extracts may be due to insulin like effect of phytochemical ingredients contained in the extracts or increase in insulin secretion from the pancreatic islets β-cells following exposure to phytochemical ingredients (Andrew, 2000). Our findings are in agreement with the findings of Jaiswal et al. (2017). During the present study, treatment of diabetic rats with combined extract was found more effective in improving body weight. Similarly, treatment of diabetic rats with mixed extract was found more effective in decreasing blood glucose level. This may be due to the synergistic antidiabetic effect of phytochemicals of both plant extracts (Isea et al., 2011). Our results support the reports of Al-Bahrani (2016), and Castellanos-Campos et al. (2016) for the hypoglycemic activity of *M. charantia*, and Adam et al. (2001) for the hypoglycemic activity of *C. colocynthis*.

During the study of liver, the level of liver-related biochemical parameters i.e., bilirubin, ALT, AST, and ALP in serum were determined at the end (day 21) of treatment (Table 3). Bilirubin, ALT, AST, and ALP are the most common liver function tests which are used commonly in clinical practice for diagnosing liver damage. ALP is a membrane-bound enzyme while ALT and AST are cytosolic enzymes. Bilirubin is associated with the function of hepatic cells. High levels of bilirubin, ALT, AST, and ALP in the blood reflect liver damage (Saleh et al., 2018). During the present study, significant increase (< 0.05) in the serum levels of these liver function markers was observed in STZ induced diabetic control rat group which indicates hepatocytes injury. Similar results have also been reported by Ramesh et al. (2007). High levels of these enzymes in the serum of STZ induced diabetic rats reflect damaged hepatocytes (Al-Bahrani, 2016; Saleh et al., 2018). Photomicrographs of liver microsections of STZ-induced diabetic control rat group showed hepatocytes with mild necrosis and infiltration (Figure 1b). During this study, STZ injection also caused renal injury, as it was apparent from the significant increase in serum urea and creatinine levels. Photomicrographs of the kidney microsections of STZ-induced diabetic control rat group showed mild epithelial desquamation and necrosis (Figure 2b). As described above that Streptozotocin especially recognizes the glucose transporter 2 receptor on β cells plasma membrane (Lenzen, 2008) and the same receptors also exists on the cell membranes of liver and kidney (Bouwens and Rooman, 2005). Therefore, STZ administration to experimental animal models could also impair the hepato-renal function (Bouwens and Rooman, 2005). The levels of bilirubin, ALT, AST, and ALP in the serum of STZ induced diabetic rats treated with the *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* mixed extract were significantly lower when compared with diabetic control rat group ($p < 0.05$). The decreasing trend in the serum levels of the above enzymes to normal values following treatment with plant extract may be due to cell membrane stability and cellular regeneration (Salam et al., 2009; Madrigal-Santillán et al., 2013). These results indicate the hepatoprotective effect of plant extracts. Photomicrographs of the liver microsections from STZ induced diabetic rat groups treated with plant extracts also showed amelioration in the liver histoarchitecture (Figure 1c-1e).

During the study of kidney, the levels of urea and creatinine in the serum of STZ induced diabetic rats treated with the *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* combined extract were significantly lower when compared with diabetic control rat group ($p < 0.05$). Photomicrographs of the kidney microsections from STZ induced diabetic rat groups treated with plant extracts showed amelioration in the renal histoarchitecture (Figure 2c-2e). These results indicate the nephroprotective effect of plant extracts in STZ induced diabetic rats. Treatment of STZ induced diabetic rats with combined plant extracts (*C. colocynthis* + *M. charantia*) was found more effective in amelioration of hepatic and renal injury. This may be due to the synergistic effect of both plant

extracts (Isea et al., 2011). Ebrahimi et al. (2016) also reported the beneficial impact of *C. colocynthis* on kidney cells. The hepatoprotective and nephroprotective effect of these plant extracts in STZ induced diabetic rat may be due to their ameliorative effect on oxidative stress. Extracts from plants contain antioxidant phytochemicals such as alkaloids, phenolic compounds, and flavonoids (Adeneye et al., 2008). Plants phenolic compounds prevent oxidative degradation of cell membrane phospholipids as their hydroxyl groups possess radical scavenging ability (Cao et al., 2019).

5. Conclusion

From the results of the present study, it was concluded that the treatment of STZ induced diabetic rats with *C. colocynthis*, *M. charantia* or *C. colocynthis* + *M. charantia* mixed extract result in body weight gain, decreased blood glucose level and amelioration of hepato-renal injury. Treatment of the diabetic rats with *C. colocynthis* + *M. charantia* mixed extract is more effective in the amelioration of diabetes and hepato-renal injuries in STZ induced diabetic male rats.

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