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Benne Lakshmi Narsimhulu
Division of Animal Biotechnology, Dept. of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India

Yarrappagaari Suresh
Division of Animal Biotechnology, Dept. of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India

Gutha Rajasekar
Division of Animal Biotechnology, Dept. of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India

Thopireddy Lavanya
Department of Zoology, Government Degree College, Kuppam, Andhra Pradesh, India

Gundala Harold Philip
Department of Zoology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India

Syed Siraj Mohiyuddin
Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

Saddala Rajeswara Reddy
Division of Animal Biotechnology, Dept. of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India

Correspondence
Saddala Rajeswara Reddy
Division of Animal Biotechnology, Dept. of Biotechnology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India

Evaluation of hepatoprotective and nephroprotective activity of methanolic extract of *Cleome viscosa* and *Cleome gynandra* in STZ-induced diabetic rats

Benne Lakshmi Narsimhulu, Yarrappagaari Suresh, Gutha Rajasekar, Thopireddy Lavanya, Gundala Harold Philip, Syed Siraj Mohiyuddin and Saddala Rajeswara Reddy

Abstract
Cleome is the largest genus in Capparaceae, with over 200 annual herb and shrub species widely circulated in pantropical and subtropical areas of the world. It is used in Ayurveda and other systems of remedy to cure several diseases, such as liver disease, chronic painful joints and mental disorders. It is used as a source of vitamin-C and iron and as a green leafy vegetable in poorer division of the peoples. To evaluate the hepatoprotective and nephroprotective activity of MeCV (methanolic extract of *Cleome viscosa*) and MeCG (methanolic extract of *Cleome gynandra*) against streptozotocin (STZ)-induced liver and kidney damage in diabetic rats. The hepatoprotective and nephroprotective activity of MeCV and MeCG were evaluated in rat serum, hepatic tissue injury markers alanine amino transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin assays were performed. The kidney injury serum markers urea and creatinine levels were also determined. Our results indicated that, oral treatment with MeCV and MeCG at dose of 400 mg/k.g b.w to diabetic rats was significantly reduced the levels of AST, ALT, ALP, total bilirubin, urea and creatinine compared with diabetic control rats. The MeCV (400 mg/k.g b.w) was showed highest hepatoprotective and nephroprotective activity the MeCV (400 mg/k.g b.w). These results suggest the potential effect of MeCV extract as a hepatoprotective and nephroprotective agent towards-STZ induced liver and kidney damage.

Keywords: *Cleome gynandra*, *Cleome viscosa*, hepatoprotective, nephroprotective, streptozotocin

1. Introduction
Diabetes mellitus is a most important endocrine disorder and emergent health problem in the majority of countries. It is a group of metabolic disorders characterized by the hyperglycemia ensuing from the deficiency of insulin production/action, or both. The chronic condition of hyperglycemia in diabetes is connected with long-term injure, dysfunction, and failure of different organs in the body, in particular eyes, kidneys, heart, nerves, and blood vessels [1]. The liver and kidney play a major function in the pathogenesis of hyperglycemia. Liver disorders like jaundice, fatty liver, cirrhosis are regularly disturbing human health in worldwide. Most important metabolic actions of body are taking place in liver [2]. Hepatic disorder generally develops in the process of elimination of toxic and injurious chemicals from the liver. Nephrotoxicity is also one of the main risk factor of drug treatment in experimental practice, regularly leading to discriminating renal failure. A lot of physiological mechanisms have been occupied in STZ-induced renal damage in diabetes [3]. In diabetes mellitus, an assortment of proteins is subjected to bonding of a sugar molecule to a protein and is consideration to add to the chronic complication of the disease.
Enhanced consideration to alternative medicines and natural remedies has encouraged new wave of investigate interest in established practices, and present require to come across for more effective agents with smaller side effects. Around 70-75% of the world's population depends on natural medicines for therapeutic diseases because they are less expenditure, less toxic, and easily available [4]. In spite of extraordinary advances in contemporary remedy no valuable medicines are accessible, which encourage liver occupations and recommend defense to the liver commencing the damage or assist to restore hepatic cells [5]. In deficiency of consistent hepatoprotective and nephroprotective drugs in modern treatment, a huge number of therapeutic preparations are suggested for the management of liver and kidney disorders [6] and fairly frequently maintained to recommend important relief. Challenges are being prepared worldwide to get scientific confirmation for these traditionally statement herbal drugs.

Cleome viscosa L. (CV) (family: Capparidaceae/ Cleomaceae), locally known as Kukkavaminta (Telugu), is an annual herb with 1mtr height. The plant is widely distributed in Bangladesh, India and Africa and south Asian countries. The roots of the plant enclose be used traditionally for the management of piles, diarrhoea, bronchitis, inflammation, liver diseases, malarial fever [7]. The leaves of the plant have also been reported to Antibacterial, antifungal and cytotoxic activities [8].

One of ethno botanically important plant, *Cleome gynandra* (Cleomaceae), a plant medicine of traditional systems of drug in India i.e., Ayurveda and siddha is worn for the management of diabetes mellitus [9]. It grows up as a wild plant in paddy grounds and also in road sides and in unwrap grass manor. In India it is never cultivated but grows up spontaneously all over the places. To the best of our knowledge there is no scientific description presented in support of the hepatoprotective and nephroprotective activity of *Cleome viscosa* and *Cleome gynandra* whole plant of methanolic extract (MeCV and MeCG) in STZ-induced diabetic rats. For that reason, to justify the established assert, we have evaluated the marker enzymes of hepatoprotective and nephroprotective effects of MeCV and MeCG using STZ-induced diabetic Wistar male rats.

2. Materials and methods

2.1 Plant collection

Healthy *Cleome viscosa* and *Cleome gynandra* whole plants were collected from Dravidian University surroundings, Kuppam, Andhra Pradesh, India. Taxonomic identification was completed by Prof N. Yasodamma, Department of Botany, S.V. University, Tirupati, India. The whole plant material was shade dried and powdered by a mechanical grinder for extraction.

2.2 Extracts preparation

The above plant powder materials were one after another extracted by using Soxhlet apparatus for 6 hrs with solvent of 1:5 ratios W/V methanol was extracted separately under the vacuum 70-80 ^{o}C. The both extracts were concentrated by using a rotary evaporator (Ro-Vap, H-Biomedical Ltd. EV311-PLUS) and subjected to freeze drying in vacuum at 35 ^{o}C-40 ^{o}C and dry powder material was obtained.

2.3 Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemicals (Bangalore, India) Company for induced diabetes. Glibenclamide, standard anti-diabetic drug (purchased from local market) was use as positive control. All other chemicals and reagents were of analytical grade and purchased from standard organizations.

2.4 Animals

We were using Male albino Wistar strain rats (180-200 g) for this study. We were maintaining the rats according to CPCSEA procedure. And the study was approved by the institutional animal ethical committee (1889/GO/Re/S/16/CPCSEA SKU/ZOO/03/2018), of Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.

2.5 Induction of diabetes

Diabetes was induced in overnight fasted experimental rats, by a single intra peritoneal injection of freshly prepared STZ (45 mg/kg/b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) as per the method followed by Rakieten *et al*. (1963). 8 h after STZ administration the rats were kept for next 24 h on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal hypoglycemia due to destruction of β-cells which in turn results into massive pancreatic insulin release. After 48 hours of STZ administration, glucose level was measured in the tail vein punctured by using an Accu-check glucometer (Roche Diagnostics Co., USA). In this study, the rats with fasting blood glucose levels (FBGL) > 270 mg/dL were considered diabetic and taken in the study. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (fasting blood glucose level ≥250 mg/dl) were selected.

2.6 Effect of acute oral administration of MeCV, MeCG and Glibenclamide on STZ-induced diabetic rats

The animals were randomly divided into five groups of six animals in each group (Total: 30 rats were taken) such as Group I - normal control; Group II - diabetic control; Group III - diabetic rats treated with glibenclamide (20 mg/kg b.w); Group IV - diabetic rats treated with MeCV (400 mg/kg b.w) and Group V - diabetic rats treated with MeCG (400 mg/kg b.w). MeCV, MeCG and glibenclamide were administered orally using oral gavage once in a day for 28 days. After 28 days of treatment, the animals were sacrificed. Blood was collected and serum was separated for the estimation of liver and kidney function markers.

2.7 Determination of liver function markers

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) was assayed by using the method of IFCC methods, 1986 [10]. The serum alkaline phosphatase (ALP) activity was estimated by the modified AMP method by using Liquid stable reagents Kit- Thomas, 1998 method [11]. The activity of serum bilirubin was measured by the method of Jendrassik, L *et al*., 1938 kit method [12].

2.8 Determination of kidney function markers

Urea in the serum was estimated by the method of Span Diagnostic kit- DAM method and serum creatinine was estimated by MOD-jaffe's kit method [13, 14].

2.9 Statistical analysis

Values were represented as mean ± SE. Data were statistically analyzed with One-way ANOVA followed by Tukey HSD test by using SPSS (version 16). P value < 0.05 was considered as significant.

3. Results

3.1 Determination of liver function markers

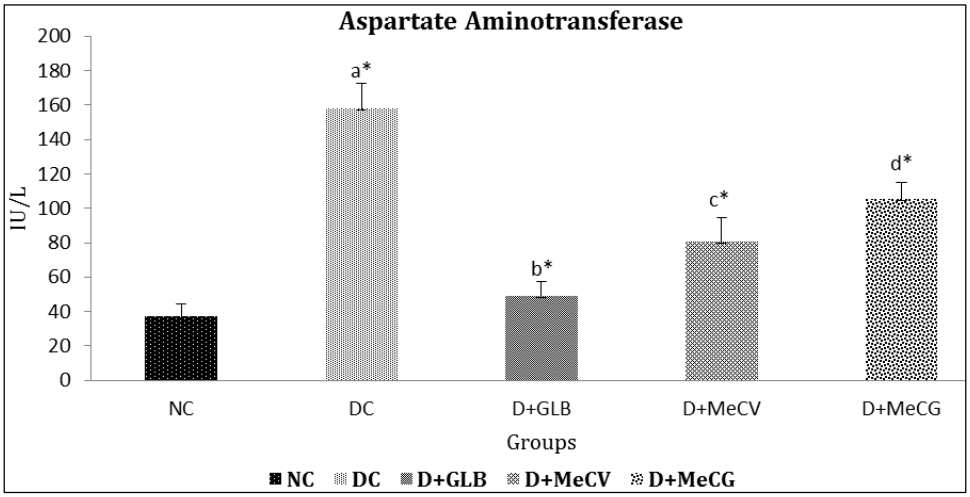

Fig 1: The effects of MeCV, MeCG and glibenclamide on alanine aminotransferase (ALT) in serum. Data were given as mean ± SE for six animals in each group. One way ANOVA is followed by *post hoc test Tukey HSD.* Values are statistically significant at $*P < 0.05$. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB, [c]D+MeCV and [d]D+MeCG treated diabetic rats were compared with diabetic control rats.

The activities of ALT, AST, ALP and total bilirubin after 28 days of oral administration of MeCV (400 mg/k.g b.w), MeCG (400 mg/k.g b.w) and glibenclamide (400 mg/k.g b.w) are represented in Figure 1, 2, 3, 4 and Table 1 and 2. As can be seen, diabetic control rats were resulted in a significantly ($p<0.05$) increased in the levels of ALT (130.83±6.03 IU/L (210.27%)), AST (158±5.94 IU/L (323.21%)), ALP (236.83±5.18 IU/L (188.82%)) and total bilirubin (5.38±0.42 IU/L (247.31%)) respectively when compared to normal control rats (ALT; 42.16±4.00 IU/L, AST; 37.33±2.81 IU/L, ALP; 82±4.67 IU/L, total bilirubin; 1.55±0.17 IU/L). Administration of MeCV (400 mg/k.g b.w), MeCG (400 mg/k.g b.w) and glibenclamide (20 mg/k.g b.w) restored the levels of ALT, AST, ALP and total bilirubin in diabetic rats.

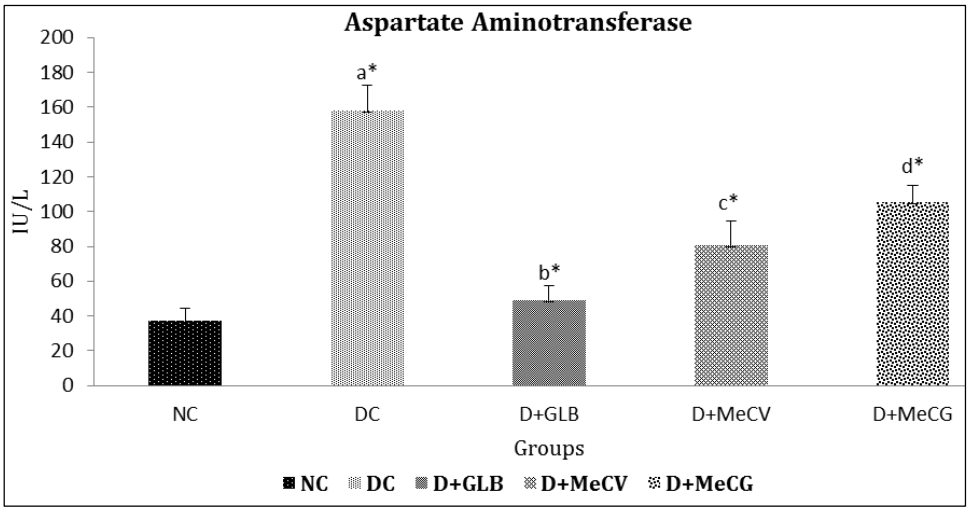

Fig 2: The effects of MeCV, MeCG and glibenclamide on aspartate aminotransferase (AST) in serum. Data were given as mean ± SE for six animals in each group. One way ANOVA is followed by *post hoc test Tukey HSD.* Values are statistically significant at $*P < 0.05$. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB, [c]D+MeCV and [d]D+MeCG treated diabetic rats were compared with diabetic control rats.

These results indicate MeCG whole plant possesses moderate antihepatotoxic activity levels in ALT; 104±1.21 IU/L (36.43% decreased), AST; 105.16±3.91 IU/L (48.94% decreased), ALP; 140.5±5.77 IU/L (55.59% decreased), and total bilirubin; 3.75±0.32 IU/L (30.34% decreased) at dose of 400 mg/kg body weight when compared to diabetic control. As shown in the MeCV at the dose of 400 mg/kg body weight has shown maximum activity been near to normal control and standard antidiabetic drug in reducing the activities of ALT; 83.16±3.29 IU/L, AST; 80.66±5.55 IU/L, ALP; 105.17±5.48 IU/L and amount of total bilirubin (2.68±0.15 IU/L) in the serum. The percentage of reduction was found to be 36.43%, 48.94%, 55.59% and 50.15%, respectively for all liver function markers, whereas the value for the glibenclamide were found to be 50.70%, 69.09%, 59.60% and 60.68%, respectively.

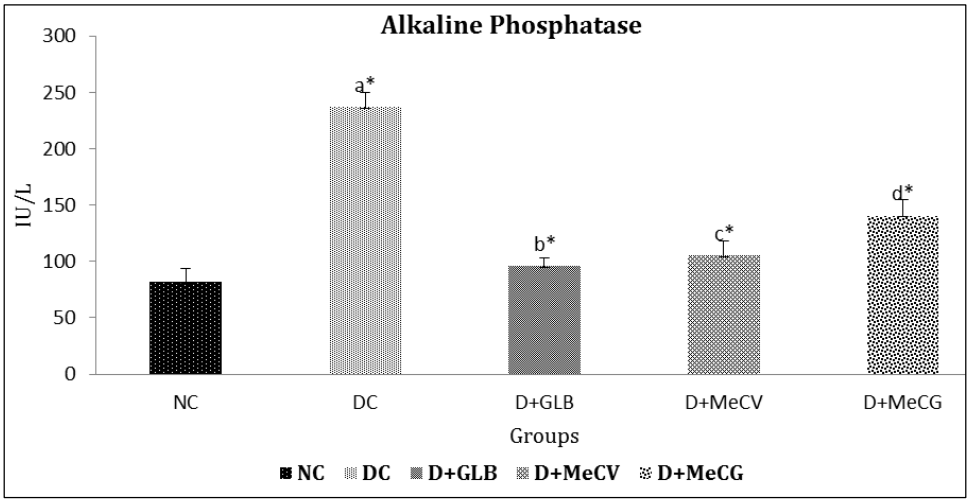

Fig 3: The effects of MeCV, MeCG and glibenclamide on alkaline phosphatase (ALP) in serum. Data were given as mean ± SE for six animals in each group. One way ANOVA is followed by *post hoc test Tukey HSD.* Values are statistically significant at $*P < 0.05$. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB, [c]D+MeCV and [d]D+MeCG treated diabetic rats were compared with diabetic control rats.

Table 1: The percentage of decreasing or increasing levels of the ALT, AST and ALP activities in STZ-induced diabetic treated rats.

Groups	% of ALT	% of AST	% of ALP
Diabetic Control (STZ-induced)	210.27▲	323.21▲	188.82▲
Diabetic+ Glibenclamide-20mg/k.g/b.w	50.70▼	69.09▼	59.60▼
Diabetic+ MeCV-400mg/k.g/b.w	36.43▼	48.94▼	55.59▼
Diabetic+ MeCG-400mg/k.g/b.w	20.50▼	33.43▼	40.67▼

▲- increased; ▼- decreased; Diabetic control rats were compared with normal control rats and remaining treated groups were compared with diabetic control group.

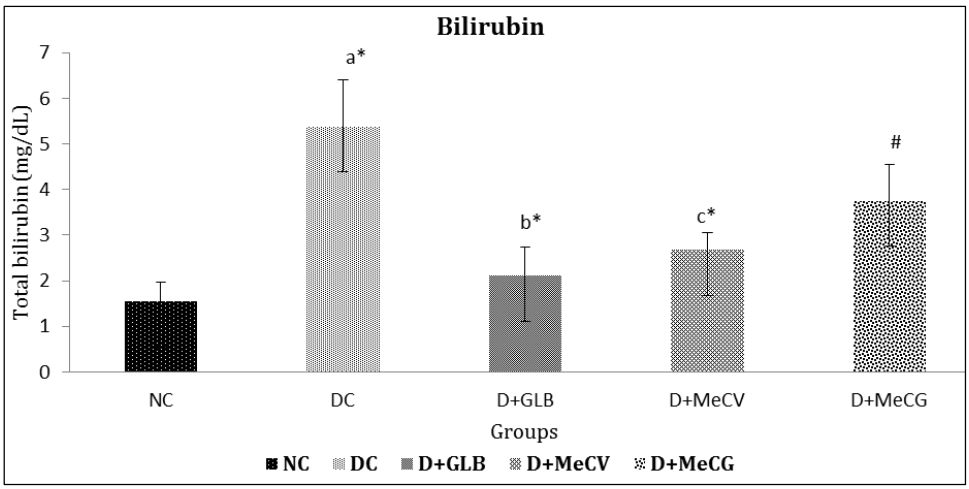

Fig 4: The effects of MeCV, MeCG and glibenclamide on total bilirubin in liver function in STZ-induced diabetic rats. Data are represented as the mean ± SE ($n = 6$). Values are statistically significant at $*P < 0.05$. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB and [c]D+MeCV treated diabetic rats were compared with diabetic control rats; # indicates there is no significance when compared with diabetic control rats.

Table 2: The percentage of decreasing or increasing levels of the total bilirubin

Groups	% of Total bilirubin
Diabetic Control (STZ-induced)	247.31▲
Diabetic+ Glibenclamide-20mg/k.g/b.w	60.68▼
Diabetic+ MeCV-400mg/k.g/b.w	50.15▼
Diabetic+ MeCG-400mg/k.g/b.w	30.34▼

▼- decreased; ▲- increased; Diabetic control rats were compared with normal control rats and remaining treated groups were compared with diabetic control group.

3.2 Determination of kidney function markers

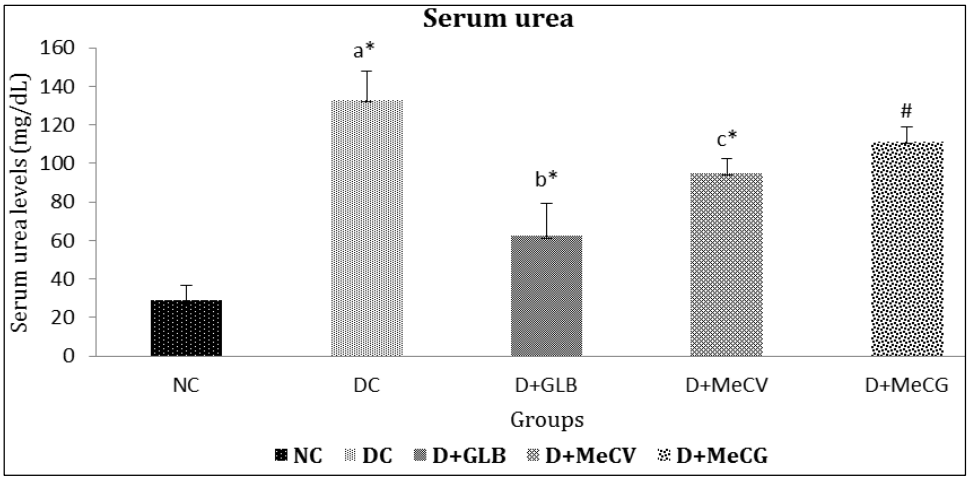

Fig 5: The effects of MeCV, MeCG and glibenclamide on serum urea in STZ-induced diabetic rats. Data are represented as the mean ± SE (n = 6). Values are statistically significant at $*P < 0.05$. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB and [c]D+MeCV treated diabetic rats were compared with diabetic control rats; # indicates there is no significance when compared with diabetic control rats.

The effect of single dose of MeCV and MeCG were studied on serum urea and serum creatinine in STZ-induced diabetic rats (Figure 5, 6 and Table 3). Kidney injury induced by STZ caused significant changed in renal markers in plasma as urea (132.83±6.06 mg/dL) by 360.69% increased and creatinine (3.38±0.32 mg/dL) by 396.82% increased respectively compared to normal control rats (28.83±3.32 mg/dL and 0.68±0.10 mg/dL). The results indicate MeCG whole plant possesses reasonable protection in urea content (111.33±3.22 mg/dL (16.18% decreased)) and creatinine (2.13±0.06 mg/dL (37.05% decreased)) at dose of 400 mg/kg body weight in diabetic rats when compared to diabetic control rats. As shown Figure 5 and 6 in the MeCV whole plant at the dose of 400 mg/kg body weight has showed highest reducing action near to normal control and glibenclamide treated rats in urea; 95±3.10 mg/dL and creatinine; 1.79±0.06 mg/dL in the serum. The percentage of urea and creatinine content decrease was found to be 47.09% and 28.48%, respectively, whereas the values for the glibenclamide were found to be 60.48% and 53.07% in the same way (Table 3).

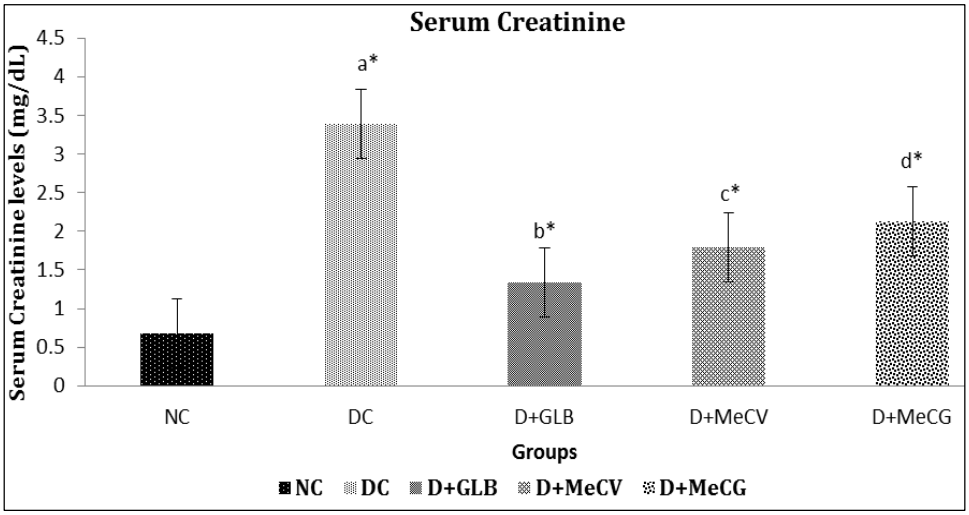

Fig 6: The effects of MeCV, MeCG and glibenclamide on serum creatinine in STZ-induced diabetic rats. Data were given as mean ± SE for six animals in each group. One way ANOVA is followed by *post hoc test Tukey HSD.* Values are statistically significant at $*P$ <0.05. [a]Diabetic control rats were compared with normal control rats; [b]D+GLB, [c]D+MeCV and [d]D+MeCG treated diabetic rats were compared with diabetic control rats.

Table 3: The percentage of decreasing or increasing levels of creatinine and urea in STZ-induced diabetic treated rats.

Groups	% of Serum urea	% of Serum creatinine
Diabetic Control (STZ-induced)	360.69▲	396.82▲
Diabetic+ Glibenclamide-20mg/k.g/b.w	53.07▼	60.48▼
Diabetic+ MeCV-400mg/k.g/b.w	28.48▼	47.09▼
Diabetic+ MeCG-400mg/k.g/b.w	16.18▼	37.05▼

▲- increased; ▼- decreased; Diabetic control rats were compared with normal control rats and remaining treated groups were compared with diabetic control group.

4. Discussion

Diabetes mellitus is linked with various complications in different organisms of the body and it is greater than ever hastily in worldwide [15]. STZ is worn for the reason that investigational treatment for diabetes research. For its discriminating β-cells of pancreatic toxicity, it is identified as a diabetes inducer in investigational animal model for understanding the manner of disease development and

circumstance that complicates something of diabetes [16]. The production of insulin in pancreas β-cells is mess up by DNA methylation by STZ consequential in poly ADP-ribose polymerase (PARP) nuclear enzyme exasperation, and as a result, NAD+ and ATP diminution is occurred. In conclusion, STZ produces of intracellular metabolism of nitric oxide that reasons DNA destruction top to necrosis of β-cells, in that way the pace of insulin production is diminished and it resulted to a systematic state known as hyperglycemia [17, 18].

Liver is the very important organ connecting in maintains the optimal blood glucose level contained by slight restrictions. Hyperglycemia induced free radical toxicity origin rigorous liver damage [19]. Moreover, bilirubin (an indicator of nonspecific cellular liver injury), AST (a distracted marker for liver injury) and ALT (a definite marker for liver parenchyma injury) are used in the assessment of liver disorders [20]. A raise of these enzyme actions replicates vigorous hepatic injure and an enhanced in the behaviors of serum ALT, AST, ALP and total bilirubin specified hepatic dysfunction and in addition liver was necrotized in STZ-induced diabetic rats [21]. ALT and AST are transaminase enzymes that play an important role in amino acids catabolism and biosynthesis and catalyse the amino transfer reactions [22]. In addition, ALP is a hydrolase enzyme which performs as distracted phosphomono esterases enzyme to hydrolyse phosphate esters [23].

The present study showed that the hepatic function markers in serum were increased in diabetic rats that consent in the companionship of the findings of Ghanbari *et al*., (2016) and Zarei *et al*., (2015) [24, 25]. In the serum, the increase of hepatic function markers might be happen as a consequence of lethal cause of hyperglycemia in the hepatic tissue of diabetic rats. An increase in ALT specify the hepatocellular injure pursued by the cardiac tissue damage and is typically go together with by get higher in AST enzyme activity. Additional, ALP is a sign of biliary function and cholestasis (reduced bile flow from liver). Levels of ALT, AST, ALP and bilirubin were increased in the in serum of diabetic rats may be for the most part due to the leakage of these enzymes from the hepatic cytosol keen on blood stream which present an indication of hepatic injury [26]. These results are in demonstration with Ramesh *et al*., (2015) [27], they reported that high levels of serum ALT, AST, ALP and total bilirubin is a familiar symptom of liver diseases and observed commonly amongst group with diabetes. On the other hand, the management of diabetic groups with MeCV and MeCG for 28 days may well improve the high activities of these enzymes. Our results are in conformity with De *et al*., (2017) [28], they confirmed that the hepatoprotective activity of methanolic extracts from *Sphaeranthus amaranthoides* and *Oldenlandia umbellate* against carbon tetrachloride induced hepatotoxicity in rat model. These results recommend that a hepatoprotective function of MeCV than MeCG in opposition to hepatic injury associated with STZ-induced diabetes.

Metabolic renal alterations are observed in investigational diabetes, which show the way to unconstructive nitrogen stability, improved the breakdown of proteins into smaller polypeptides or amino acids and lesser protein synthesis [29]. Alterations in protein metabolism incorporate a reduced uptake of amino acids by tissues, an elevated rate of breakdown of proteins into smaller polypeptides or amino acids and a drop in protein fusion leading to an enhance in the production of urea by the liver [30]. The high level of urea, glucose and further compounds in the kidney occurs from the enlarged glycation of blood proteins can generate vascular alterations in the system of renal and injure the kidney and thus promote a defeat of protein in the urine [31].

Diabetic nephropathy is a familiar along with serious problem where kidneys are injured in addition to fails to function. In the present study, the STZ induced diabetic rats are connected with significant increase in the levels of urea and creatinine are demonstrating damage renal function. The diabetic hyperglycemia stimulates increase of the levels of serum urea and creatinine which are measured as significant markers of renal dysfunction [32]. Urea is the end product of protein catabolism in the living organization. They are produce in the liver from ammonia, formed as a product of the de-amination of amino acids [33]. The quantity of urea in blood was high in diabetic condition as an end result of enlarged proteolysis in blood and tissues owing to harmful nitrogen stability which is connected with falling the protein synthesis and also the increased oxidative stress stimulate the increase in the levels of urea and creatinine [34].

Serum creatinine absorption is used as biochemical investigative markers to evaluate the destruction of renal function and drug-induced toxicity in experimental practice [35]. Creatinine is a derivative of phosphocreatine and creatine; these are measured as power storage complexes in muscle. The changeable deliberation of creatinine is not only used to assess the lack of kidney function, while used to distinguish and the management of linked toxic assets of drugs in the kidney of investigational rats [36]. In the present study there is a significant increase in the creatinine levels in STZ-induced diabetic rats and these outcomes are in similar with those of Siboto *et al*., (2018) [37] and Ashraf *et al*., (2013) [38] in that they suggested the methanolic extract of *Momordica balsamina* and aqueous extract of *Artemisia afra* showed the same possessions on kidney function parameters. In the present investigation, the management of MeCV and glibenclamide to diabetic rats upturned the distorted levels of serum urea and creatinine to near normal level, which designates the renoprotective function of MeCV in diabetic nephropathy.

5. Conclusion

The result of the present study specifies that the MeCV possess good protective belongings than the MeCG against hepatic and renal injury in STZ-induced diabetic experimental rats. The hepatoprotective and nephroprotective effects of MeCV are confirmed by the diminution in hepatic function markers and renal function markers in the diabetic treated rat's blood serum. From this investigation, we concluded that MeCV is an effective antihyperglycemic manager that can avoid the improvement of diabetic complications like hepatotoxicity and nephropathy.

6. Acknowledgement

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