INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ENZYMATIC AND NONENZYMATIC ANTIOXIDANT ACTIVITIES OF *Cleome viscosa* AND *Cleome gynandra* WHOLE PLANT METHANOLIC EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Benne Lakshmi Narsimhulu [1], Yarrappagaari Suresh [1], Gutha Rajasekar [1], Thopireddy Lavanya [2], Syed Siraj Mohiyuddin [3], Saddala Rajeswara Reddy [1]*
[1]Division of Animal Biotechnology, Dept of Biotechnology, School of Herbal studies and Naturo Sciences, Dravidian University, Kuppam, A.P, India
[2]Dept of Zoology, Government Degree College, Kuppam, A.P, India
[3]Division of Toxicology, Department of Zoology, S.V. University, Tirupati, Andhra Pradesh, India
*Corresponding Author Email: drrajeswarareddy@dravidianuniversity.ac.in

Article Received on: 24/05/18 Approved for publication: 22/06/18

DOI: 10.7897/2230-8407.096101

ABSTRACT

This study was designed to scrutinize the enzymatic and nonenzymatic antioxidant activities of the methanolic extract of whole plant of *Cleome viscosa* and *Cleome gynandra* (MeCV and MeCG) in streptozotocin (STZ) induced diabetic rats. Oral administration of MeCV, MeCG and Me.CV+CG combination at a dosage of 400 mg/ kg b.w for 28 days on diabetic rats. The enzymatic and non-enzymatic antioxidant assays were measured by using standard procedures in liver tissue of diabetic and normal rats. There is a significant ($p < 0.05$ and $p < 0.01$) improvement in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione - s-transferase (GST) in liver tissue of STZ induced diabetic treated rats when compared with the untreated diabetic rats and the contents of ascorbic acid (AA) and glutathione (GSH) levels were also significantly increased in the treated diabetic rats when compared with untreated diabetic rats. These results clearly indicated that the whole plants of MeCV have good hepato protective activity than MeCG whole plant in STZ induced diabetic rats.

Keywords: Streptozotocin, Liver damage, *Cleome viscosa, Cleome gynandra,* Antioxidant activity and superoxide dismutase.

INTRODUCTION

Diabetes mellitus (DM) is acknowledged as one of the major health problem in the world[1]. DM is a group of metabolic disorders manifest in the occurrence of high concentration of the glucose in blood because of the inappropriate production of insulin commencing the pancreas or immobility of pancreas β-cells to the insulin[2]. Diabetes mellitus have the prospective to cause chronic morbidity due to the complications to various organs of the body, related with microvascular injure and neuropathy affecting the renal, retina, peripheral nerves and peripheral arteries[3]. The living system is equipped with different antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)) and nonenzymatic antioxidants like ascorbic acid and glutathione (GSH) play a key role in the scavenging of free radicals and defending the cell membrane from injury[4]. Therefore, antioxidants help to reducing the risk of oxidative damages in diabetic and hyperlipidaemia patients. The main sources of antioxidants are medicinal plants[5]. In predictable remedial practice, the current treatment of diabetes mellitus is statement to have side effect. Several oral therapeutic drugs are the main choice of type 2 diabetes treatments. The glucose lowering drugs include insulin secretion (sulfonylurea and meglitinides), insulin sensitizers (metformin, biguanides and thiazolidinediones) and α- glucosidase inhibitors (acarbose and miglitol)[6]. The aim of person's oral hypoglycaemic drugs is to remodel the fundamental metabolic disorder, correlated to poor insulin conflict, insulin emission, and improved hepatic gluconeogenesis.

However, these diabetic treated oral drugs have restricted effectiveness and sometimes formed several side effects like weight increase, hypoglycemia, liver damage, canal disturbances, cardiopathy, and abdominal[7, 8]. Therefore, because of the side effects linked with the present antidiabetic drugs, there are needed to develop effectual, protected and low-priced drugs for diabetes management. Such effectual, protected and low-priced drugs could be acquired by medicinal plants which have been used by humans to prevent the diseases together with diabetes since the day of improvement[9].

Cleome viscosa L. belongs to the family Capparidaceae (presently Cleomaceae) is a broadly distributed aromatic plant with yellow flowers and long slim shells have seeds. The whole plant is sticky in nature and 1 miter height. It is originating throughout the India, obtained in waste places and road sides. In Telugu it is known as "Kukkavamita" in Indian traditional medicine. Usually, this plant is used to treat fever, diarrhoea, bronchitis, inflammation, liver diseases, malarial fever and skin diseases[10]. The leaves juice is useful in piles, earache and lumbago. *Cleome Viscosa L.* leaves extract used as a hepatoprotective agent against Carbon tetrachloride induced toxicity[11]. Literature survey specifies that no studies have been conceded on the experimental estimation of hepatoprotective effect of *Cleome Viscosa L.* whole plant against STZ-induced diabetes.

Cleome gynandra commonly known as spider flower in English belongs to the family capparidaceae (presently Cleomaceae). It is one of the basic foods in most of the African region. It has a wide

range of medicinal uses and used in traditional systems of medicine all over the world. *C.gynandra* has many scientifically proven medicinal properties such as anthelmintic and anti microbial, antioxidant and anti-inflammatory activity[12]. The aim of the present work is to investigate the hepato protective activity of *Cleome viscosa* and *Cleome gynandra* whole plant of methanolic extract against stz-induced diabetic rats.

MATERIALS AND METHODS

Plant collection

The whole plants of *Cleome viscosa* and *Cleome gynandra* were collected from Dravidian University surroundings, Kuppam, Andhra Pradesh, India. Taxonomic identification was made 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.

Extracts preparation

The above both plant powder materials were successively extracted by using Soxhlet apparatus for 6 hrs with methanol solvent (1:5 ratio W/V) was extracted independently under the vacuum 70-80^0 c concentration. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in vacuum at 35^{0}c-40^{0}c and dry powder was obtained.

Animal ethical committee approval

The study was getting approval from Animals Ethical Committee of Sri Krishnadevaraya University, Andhra Pradesh, India. (1889/GO/Re/S/16/CPCSEA-SKU/ZOO/03/2018) to perform the animal work.

Animals

In this present study, healthy Wistar strain male albino rats weighing approximately 180–200 g were used during the study. All animals were kept in polyethylene cages and maintained in animal house under the standard condition 24°C ± 2°C of temperature with a 12:12 light: dark cycle. The animals were fed with stranded pellet diet and water.

Induction of Diabetes Mellitus

Diabetes was induced in healthy male Wistar Albino rats with body weights ranging from 180 to 200 g, by a single intra peritoneal injection (I.P) of freshly prepared 45 mg/kg/b.w of Streptozotocin (STZ) was dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12–15 hour as per the method followed by Rakieten et al. (1963)[13]. After 8 h of STZ administration the rats were kept for next 24 hours on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of generate fatal hypoglycemia due to destruction of β-cells which in turn consequences into immense pancreatic insulin release. Diabetes was assessed by determining the fasting blood glucose levels after 48 hours of STZ injection. The blood glucose levels in STZ rats were increased to markedly higher levels than normal rats. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (fasting blood glucose level ≥250 mg/dl) were selected.

Study design

Total thirty six rats were used in the study. Animals were divided into six groups (n=6).

Group-I: Normal control (untreated but having free access to water and feed).
Groups-II: Diabetic control (did not received any treatment and served as negative control)
Group-III: Diabetic+Glibenclamide-20 mg/kg b.w (orally once daily respectively for 28 days and conceder as positive control)
Groups-IV: Diabetic+MeCV-400 mg/kg b.w (orally once daily respectively for 28 days)
Groups-V: Diabetic+MeCG-400 mg/kg b.w (orally once daily respectively for 28 days)
Groups-VI: Diabetic+Me.CV+CG-400 mg/kg b.w (orally once daily respectively for 28 days).

Preparation of tissue homogenates

Liver tissue were isolated from rat after sacrificing them and immediately stored in ice cold phosphate buffer saline (10 mmol/L, pH 7.4.). 10 mmol/L of ice cold phosphate buffer saline (pH 7.4) was used to prepare 25% homogenate, then it was centrifuged at 4, 000 rpm for 10 min at 4°C for to wash out cellular debris and supernatant was collected and stored at -80°C till quantification of antioxidant enzymes and nonenzymatic antioxidant assays.

Estimation of Antioxidants

Enzymatic antioxidants

Determination of Superoxide Dismutase activity

Superoxide dismutase (SOD) was scrutinized according the method of Misra and Fridovich[14]. Reaction mixture contains 1 mL of sodium carbonate (50 mM), 0.4 mL of nitroblue tetrazolium (25 μm) and freshly prepared 0.2 mL of hydroxylamine hydrochloride (0.1 mM). The reaction mixtures were mixed by inversion follow by the addition of a clear supernatant of 0.1 mL of liver homogenate (1:10 w/v). The change in absorbance of tested samples was recorded at 560 nm.

Estimation of Catalase activity

Catalase was evaluating calorimetrically at 620 nm and expressed as moles of H_2O_2 consumed/min/mg protein as described by Sinha (1972)[15]. The 1.5 mL of reaction mixture contained 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 mL of 1:3 ratio dichromate-acetic acid reagent (5% potassium dichromate and glacial acid were mixed).

Determination of Glutathione-S-Transferase (GST) activity

The activity of GST was determined according to the method of Habig et al (1974)[16]. Briefly, 0.1 mL of 1-Chloro-2, 4-dinitrobenzene (CDNB) solution was pipetted out into a conical flask before adding the 1 mL of phosphate buffer and 1.7 mL of distilled water. Subsequently, the reaction mixture was incubated at 37°C for 5 min. The 0.1 mL of tissue homogenate and 0.1 mL of Glutathione reduced (GSH) solution were added by using a micropipette after the incubation. Absorbance was recorded at 340 nm were taken for 5 min at 60 s interval using a UV-Vis spectrophotometer.

Nonenzymatic antioxidants

Estimation of Ascorbic acid

Hepatic vitamin C levels were determined by the using method of Jacques-Silva et al (2001)[17]. Protein of liver was homogenate by

using 10% of cold trichloroacetic acid (TCA) solution (1:1 v/v) and proposition to centrifugation again. This supernatant was then used for estimation. A 300 mL of aliquot sample in a 575 mL of final volume was incubated for at 37^0C for 3 h. 500 mL of 65% of H_2SO_4 (v/v) was added to the reaction medium. The medium was determined by a color reagent containing of 4.5 mg/mL of dinitrophenyl hydrazine (DNPH) and 0.075 mg/ mL of $CuSO_4$. Ascorbic acid levels are expressed as mg/g tissue.

Estimation of Glutathione (GSH)

Glutathione (GSH) activity was determined by the procedure of Carlberg and Mannervik (1985)[18]. The assay solution contained BSA (10%), Phosphate buffer (50 mM, pH = 7.6), NADPH (2 mM), 20 mM of Glutathione oxidized (GSSG). Absorbance was measured at 340 nm at a temperature of 25°C. The Glutathione (GSH) activity was expressed in U/gm tissue.

Statistical analysis

The experimental results were expressed as mean ± SD (n=6). The results were analyzed by using one-way analysis of variance and the group means were compared by Tukey's HSD (SPSS version 16). A differentiation was measured to be statistically significant when the p-value is lower than $p < 0.05$ and $p<0.01$.

RESULTS

Enzymatic antioxidants

Determination of Superoxide dismutase activity (SOD)

The activity of the liver Superoxide dismutase enzyme in diabetic treated rats and untreated diabetic rats with MeCV, MeCG and combination of both plants Me.CV+CG is summarized in figure 1. The effect of 400 mg/kg b.w. of MeCV (18.0+0.06) treated diabetic rats was more effective than 400 mg/kg b.w. of MeCG (17.58+ 0.09) and 400 mg/kg b.w. of Me. CV+CG combination (17.68+0.13) treated diabetic rats. There was a higher in the activity of Superoxide dismutase in the normal rats (19.7+0.06) significantly ($p<0.05$ and $p<0.01$) when compared to non treated diabetic rats (6.3+ 0.49). Treatment with the extracts in diabetic rats increased the SOD enzyme activity but, the increase level was lower when compared to the glibenclamide treated diabetic rats (18.23+0.10).

Estimation of Catalase activity (CAT)

As shown in figure 2, STZ-induced diabetic control rats (4.68+ 0.18 Units/mg protein) showed a significant ($p<0.05$ and $p<0.01$) decrease in catalase enzyme antioxidant activity when compared to the normal control rats (9.96+0.32 Units/mg protein). Treatment with 400 mg/kg b.w. of MeCV (6.04+0.23 Units/mg protein), 400 mg/kg b.w. of MeCG (5.42+0.10 Units/mg protein) and the combination of both plants 400 mg/kg b.w. of Me.CV+CG (5.72+0.07 Units/mg protein) for 28 days produced a significant increase in the activity of catalase enzyme compared to non treated diabetic rats. The activity of the catalase enzyme in the MeCV (Group-IV) treated diabetic rats was similar to the glibenclamide (8.56+0.35 Units/mg protein) treated diabetic rats (Group-III).

Determination of Glutathione-S-transferase (GST)

Figure 3 shows the GST activity in liver of normal rats and diabetic rats treated with MeCV, MeCG and Me.CV+CG. The results of the present study showed decreased in the level of GST in untreated diabetic rats (118.80+41.49 U/mg protein) when compared to normal rats (831.35+ 23.33 U/mg protein). The 20 mg/kg/b.w of glibenclamide treated diabetic rats showed about four fold increase in GST activity (531.25+ 32.77 U/mg protein) when compared with diabetic control rats. MeCV and Me.CV+CG treated groups (341.75+ 23.55 and 256.35+ 26.90 U/mg protein) showed significant increase in the GST level when compared to diabetic control rats. MeCG treated group showed no change in GST level (143.75+ 39.03 U/mg protein) in comparison with diabetic control rats.

Nonenzymatic antioxidants

Estimation of Ascorbic acid

Figure 4 shows the hepatic ascorbic acid (vitamin C) content in all experimental groups. As can be seen, in liver vitamin C content was significantly reduced in the diabetic control rats (30.48±0.58 µg/mg of protein) when compared to the normal control rats (85.61± 3.89 µg/mg of protein). However, the treatment with 400 mg/k.g/b.w of MeCV (52.20± 1.91 µg/mg of protein) have high in the hepatic vitamin C content than remaining treated groups of 400 mg/k.g/b.w of MeCG (40.05± 2.23 µg/mg of protein) and 400 mg/k.g/b.w of Me.CV+CG (47.89±0.81 µg/mg of protein) vitamin C content in the diabetic treated rats when significantly ($p< 0.05$ and $p<0.01$) compared to diabetic control rats. Glibenclamide treated rats (63.01± 1.12 µg/mg of protein) were significantly similar to normal control rats when compared to diabetic control rats.

Estimation of Glutathione (GSH)

Figure 5 shows the hepatic GSH content in experimental treated and untreated diabetic rats. The decreased GSH levels (11.57±0.76 µg/gm of Tissue) were observed in non treated diabetic control rats when compared to normal rats. After 28 days of the 400 mg/k.g/b.w of MeCV, MeCG and Me.CV+CG administration of diabetic rats and 20 mg/k.g/b.w of glibenclamide treated rats have shown 14.16± 0.92, 12.97± 0.41, 13.70± 0.29 and 16.93± 0.75 µg/gm of Tissue respectively were significantly ($p<0.05$ and $p<0.01$) increases when compared with diabetic rats (Group-II).

DISCUSSION

Diabetic and experimental animal model show signs of high oxidative stress due to constant and chronic hyperglycemia, which in this manner reduces the system of antioxidative defence activity, as a result promote the beginning of free radical production[19]. Alterations in the antioxidant status results in imbalance of enzymes such as SOD, catalase, GSH-Px and GST[20]. SOD and catalase enzymes are considered as major enzymes because they are implicated in the direct removal of ROS[21]. The present study was carried out to assess the enzymatic and nonenzymatic antioxidant property of the MeCV, MeCG and both plants combination of Me.CV+CG in STZ-induced diabetic oxidative stress in liver tissue. It is the first comprehensive report on liver enzymatic and nonenzymatic antioxidant activity of MeCV and MeCG in STZ-induced diabetic rats. In addition MeCV and MeCG activity was compared with glibenclamide oral hypoglycemic agent.

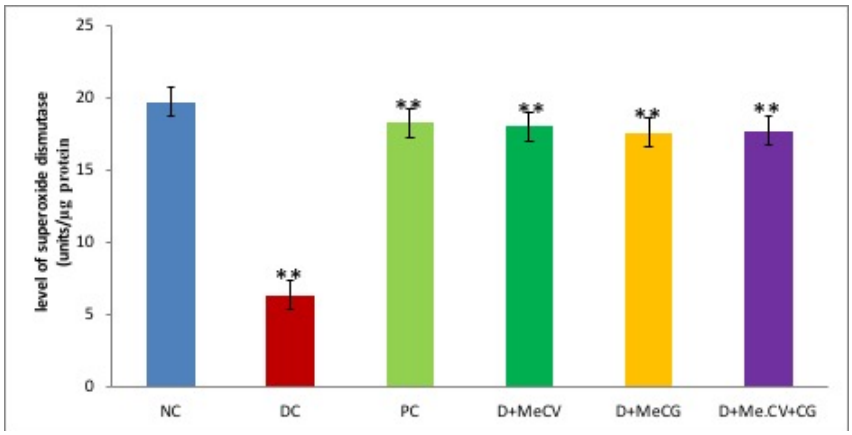

Figure 1: Effect of *Cleome viscosa* and *Cleome gynandra* whole plant of methanolic extract on the activity of superoxide dismutase (SOD) antioxidant enzyme in STZ- induced diabetic treated rats.

Values are given as mean± SD for each group have six rats. ** p< 0.01 Values are statistically significant. Group-2 was compared with Group-1; Group-3 (Diabetic+ Glibenclamide 20 mg/k.g/b.w treated), Group-4 (Diabetic+MeCV-400 mg/k.g/b.w treated), Group-5 (Diabetic+MeCG-400 mg/k.g/b.w treated) and Group-6 (Diabetic+ Me-CV+CG-400 mg/k.g/b.w treated) were compared with Group-2 (Diabetic control) was analyzed by One-way ANOVA (Tukey HSD test).

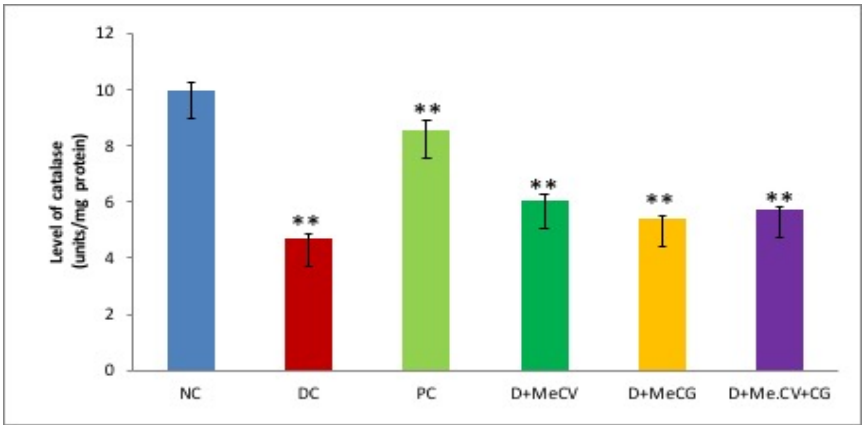

Figure 2: Effect of *Cleome viscosa* and *Cleome gynandra* whole plant of methanolic extract on the activity of catalase (CAT) antioxidant enzyme in STZ- induced diabetic treated rats.

Values are given as mean± SD for each group have six rats. ** p< 0.01 Values are statistically significant. Group-2 was compared with Group-1; Group-3 (Diabetic+ Glibenclamide 20 mg/k.g/b.w treated), Group-4 (Diabetic+MeCV-400 mg/k.g/b.w treated), Group-5 (Diabetic+MeCG-400 mg/k.g/b.w treated) and Group-6 (Diabetic+ Me-CV+CG-400 mg/k.g/b.w treated) were compared with Group-2 (Diabetic control) was analyzed by One-way ANOVA (Tukey HSD test).

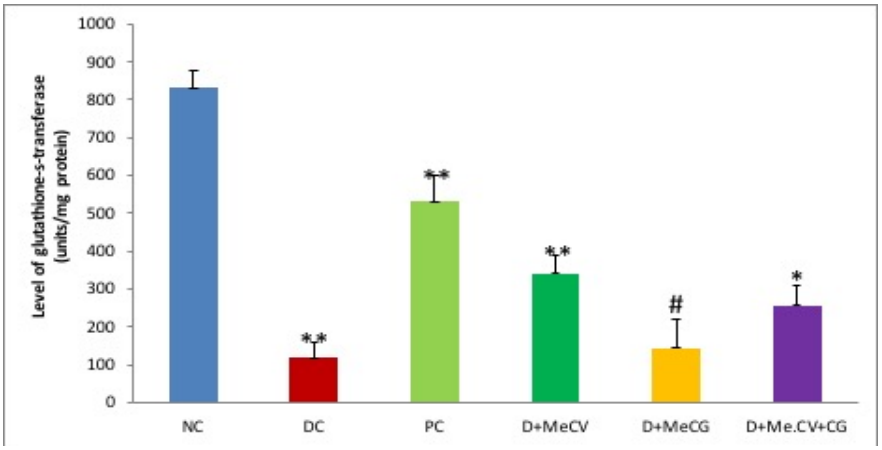

Figure 3: Effect of *Cleome viscosa* and *Cleome gynandra* whole plant of methanolic extract on the activity of glutathione-s-transferase (GST) antioxidant enzyme in STZ- induced diabetic treated rats.

Values are given as mean± SEM for each group have six rats. * p < 0.05 and ** p < 0.01 Values are statistically significant. # indicates there is no significance. Group-2 was compared with Group-1; Group-3 (Diabetic+ Glibenclamide 20 mg/k.g/b.w treated), Group-4 (Diabetic+MeCV-400 mg/k.g/b.w treated), Group-5 (Diabetic+MeCG-400 mg/k.g/b.w treated) and Group-6 (Diabetic+ Me-CV+CG-400 mg/k.g/b.w treated) were compared with Group-2 (Diabetic control) was analyzed by One-way ANOVA (Tukey HSD test).

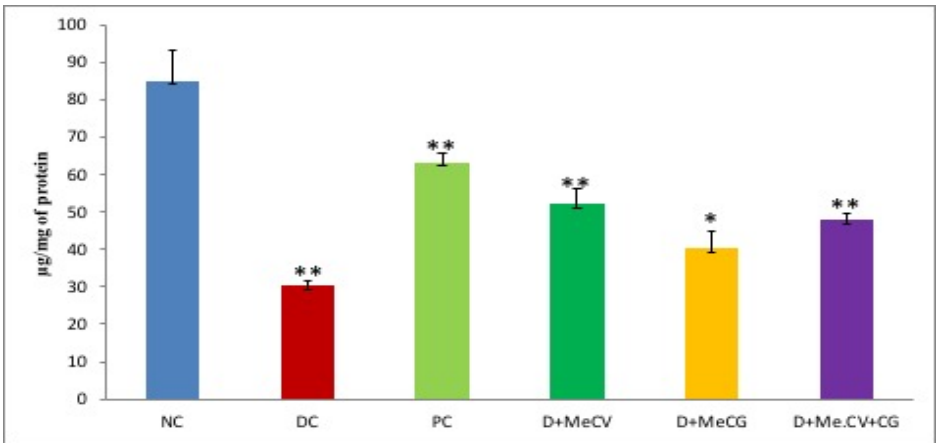

Figure 4: Effects of *Cleome viscosa* and Cleome gynandra whole plant of methanolic extract on ascorbic acid levels of liver tissue in diabetic experimental rats.

Values are given as mean± SEM for each group have six rats. * $p < 0.05$ and ** $p < 0.01$ Values are statistically significant. Group-2 was compared with Group-1; Group-3 (Diabetic+ Glibenclamide 20 mg/k.g/b.w treated), Group-4 (Diabetic+MeCV-400 mg/k.g/b.w treated), Group-5 (Diabetic+MeCG-400 mg/k.g/b.w treated) and Group-6 (Diabetic+ Me-CV+CG-400 mg/k.g/b.w treated) were compared with Group-2 (Diabetic control) was analyzed by One-way ANOVA (Tukey HSD test).

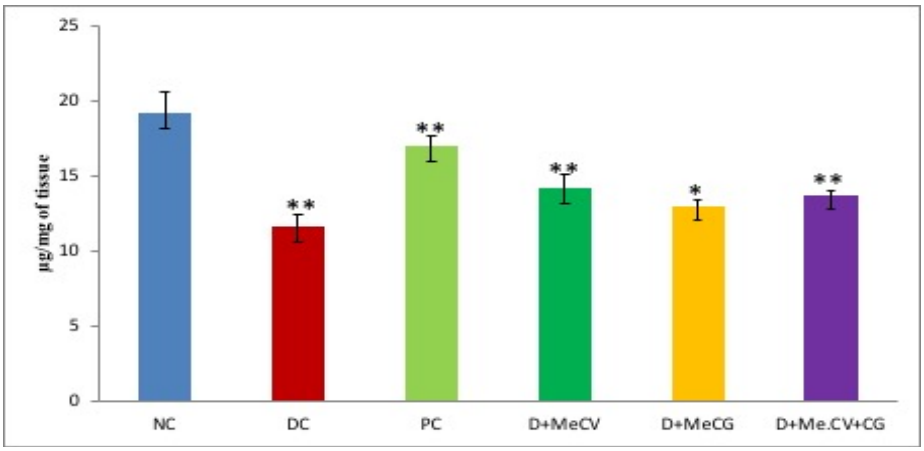

Figure 5: Effects of *Cleome viscosa* and Cleome gynandra whole plant of methanolic extract on glutathione (GSH) levels of liver tissue in diabetic experimental rats.

Values are given as mean± SEM for each group have six rats. * $p < 0.05$ and ** $p < 0.01$ Values are statistically significant. Group-2 was compared with Group-1; Group-3 (Diabetic+ Glibenclamide 20 mg/k.g/b.w treated), Group-4 (Diabetic+MeCV-400 mg/k.g/b.w treated), Group-5 (Diabetic+MeCG-400 mg/k.g/b.w treated) and Group-6 (Diabetic+ Me-CV+CG-400 mg/k.g/b.w treated) were compared with Group-2 (Diabetic control) was analyzed by One-way ANOVA (Tukey HSD test).

Superoxide dismutase (SOD) is the primary procession of defence against oxidative stress. Its main function is, to catalyse the exchange of superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2) and for this reason moderate the toxic cause owing to this fundamental or additional free radicals derivative from secondary response[22]. The H_2O_2 produced by SOD is eliminating as water, from the activity of catalase and GSH-Px, thus defensive the body as of oxygen toxicity[23]. Catalase and SOD are the two major antioxidant enzymes to remove the ROS *in vivo*. Hence, in the present study activities of SOD, CAT and GST (enzymatic antioxidants) and ascorbic acid (Vitamin C) and Glutathione (GSH) are nonenzymatic antioxidants were evaluated in liver tissue of diabetic and non diabetic rats.

In the present study activities of SOD, catalase and GST levels were reduced in diabetic rat liver when compared with normal rats, signifying abnormalities in antioxidant protective system which could be owing to free radical induced inactivation or glycation of the enzyme in diabetic stress[24]. Schettler et al (1994)[25] suggested that the decreased antioxidant production is owing to enlarged oxygen metabolites causes diminish in the action of the antioxidant defence structure. Majority of the authors reported the decreased enzymatic antioxidant activity in STZ induced diabetic rats[26-28].

On the other hand, our results confirmed that the treatment with MeCV (400 mg/kg b.w), MeCG (400 mg/kg b.w) and both plant combination of Me.CV+CG (400 mg/kg b.w) were increased in SOD, CAT and GST enzyme activities in liver of STZ-induced diabetic rats (Figures. 1, 2, and 3). When compared to MeCG and both plant combination of Me.CV+CG, MeCV has shown high levels of enzyme activity. Based on these findings, we can suggest that MeCV is capable to alter in all SOD, CAT and GST enzymatic antioxidant activities, which may be incredibly significant since it increases the scavenging ability of ROS of liver tissue provided that elevated defense against oxidative damage by diabetes in these tissues. The same occurrence was seen in the results of glibenclamide treated group. In similar results were observed in previous studies Rajasekar et al (2014)[29], Saleem et al (2014)[30] and Vijayaraj and Sri Kumaran (2018)[31].

Ascorbic acid (Vitamin C) is a most important antioxidant to essential for the scavenging of toxic free radicals in both plasma and tissue[32]. It is acts as a co-antioxidant by regenerate the

vitamin-A, vitamin-E and glutathione (GSH) from radicals. Vitamin E is a fat soluble vitamin; regeneration of vitamin E requires ascorbic acid, an aqueous phase antioxidant, which requires GSH[33]. A decreased level of ascorbic acid and GSH in liver of diabetic rats reported in the present study might be owing to its improved use and deactivation of ROS. Mirzaei, Rezaei, and Salehpour. (2016)[34] and Subramani and Leelavinothan. (2012)[35] reported similar results in the toxic rabbit and diabetic rat respectively. At that same time Oral administration of MeCV improved the level of ascorbic acid and GSH levels in liver of STZ induced diabetic rats compared to the whole plant of MeCG, it indicates the directly scavenging the free radicals which in turn decreased oxidative stress. This activity might be due to free scavenging and antioxidant properties of MeCV. Similar results have been obtained by treatment with hydroethanolic extract of *Senna alata* in the STZ induced diabetic rat liver tissue[36].

CONCLUSION

The present study, whole plant of MeCV in the dose of 400 mg/ kg/ b.w has improved the enzymatic (SOD, catalase and glutathione-s-transferase) and nonenzymatic (ascorbic acid and glutathione) antioxidant levels significantly compared to 400 mg/ kg/ b.w of whole plant of MeCG. Based on these results we concluded that whole plant of MeCV possesses hepatoprotective activity. Therefore, further investigations are needed to clarify the exact mechanism by which MeCV exhibit its therapeutic properties.

ACKNOWLEDGEMENT

The authors are thankful to their supportive in completion of this research work.

REFERENCES

1. Rao S, Mohan K, Srinivas P. Evaluation of anti-diabetic activity of *Hydnocarpus laurifolia* in streptozotocin induced diabetic rats. Asin J Pharm Clin Res 2014; 7:62-4.
2. Shyam T, Ganapaty S. Evaluation of antidiabetic activity of methanolic extracts from the aerial parts of *Barleria montana* in streptozotocin induced diabetic rats. Journal of Pharmacognosy and Phytochemistry 2013; 1 (May): 187-192.
3. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice 2011; 94 (December):311-21.
4. Poongothai K, Ponmurugan P, Ahmed KS, Kumar BS, Sheriff SA. Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine 2011; 4 (October):778-85.
5. Peng KT, Hsu WH, Shih HN, Hsieh CW, Huang TW, Hsu RW, *et al*. The role of reactive oxygen species scavenging enzymes in the development of septic loosening after total hip replacement. J Bone Joint Surg Br 2011; 93 (September):1201-9.
6. Gupta R, Sharma AK. Anti-Hyperglycemic Activity of Aqueous Extracts of Some Medicinal Plants on Wistar Rats. J Diabetes Meta b 2017; 8: 752-7.
7. Wright Jr. E, Scism-Bacon JL, Glass LC. Oxidative stress in type 2 diabetes: The role of fasting and postprandial glycaemia. International Journal of Clinical Practice 2006; 60 (March): 308–314.
8. Ceriello A, Quagliaro L, Catone B, Pascon R, Piazzola M, Bais B, *et al*. Role of hyperglycemia in nitro tyrosine postprandial generation. Diabetes Care 2002; 25: 1439–1443.
9. Surendran S, Eswaran MB, Vijayakumar M, Rao CV. *In vitro* and *in vivo* hepatoprotective activity of *Cissampelos pareira* against carbon tetrachloride induced hepatic damage. Indian Journal of Experimental Biology 2011; 49: 939-945.
10. Raghavan R, Renuka S, Dheeba B, Sivakumar R. *In vivo* investigation of hepatoprotective activity of *Cleome viscosa L*. in albino rats. Der Pharmacia Lettre 2016; 8:308-313.
11. Nishant KG, Vinod KD. Evaluation of hepatoprotective activity of *Cleome viscosa* Linn. Extract. Indian J Pharmacol 2009; 41:36-40.
12. Shanmuganathan T, Karthikeyan AVP. Phytochemical Analysis and Antioxidant Activities of *in vivo* and *in vitro* Shoots of *Cleome gynandra L*. International Journal of Science and Research 2016; 5:208-211.
13. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37919). Cancer Chemother Rep 1963; 29:91–8.
14. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem 1972; 247: 3170–3175.
15. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389–394.
16. Habig WH, Pabst MS, Jekpoly WB. Glutathione transferase: A first enzymatic step in mercaptuiric acid formation. J. Biol. Chem 1974; 249: 7130–7139.
17. Jacques-Silva MC, Nogueira CW, Broch LC, Flores EMM, Rocha JBT. Diphenyl diselenide and ascorbic changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacol Toxicol 2001; 88 (March):119-25.
18. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985; 113: 484–490.
19. Mohammad A, Laxmi V, Mohammad HAK, Salman M, Mehar N, Saima W, *et al*. Neuromodulatory Effects of Hesperidin in Mitigating Oxidative Stress in Streptozotocin Induced Diabetes. BioMed Research International 2014; 249031:1-9.
20. Subash-Babu P, Alshatwi AA, Ignacimuthu S. Beneficial Antioxidative and Antiperoxidative Effect of Cinnamaldehyde Protect Streptozotocin-Induced Pancreatic b-Cells Damage in Wistar Rats. Biomolecules & Therapeutics 2014; 22: 47-54.
21. Yubero-Serrano EM, Delgado-Lista J, Peña-Orihuela P, Perez-Martinez P, Fuentes F, Marin C, *et al*. Oxidative stress is associated with the number of components of metabolic syndrome: LIPGENE study. Experimental & Molecular Medicine 2013; 45:1-7.
22. Yang XW, Liu FQ, Guo JJ, Yao WJ, Li QQ, Liu TH, *et al*. Antioxidation and anti-inflammatory activity of Tang Bi Kang in rats with diabetic peripheral neuropathy. BMC Complementary and Alternative Medicine 2015; 15: 1-10.
23. Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic Stem Extract of *Coscinium fenestratum* Regulates Carbohydrate Metabolism and Improves Antioxidant Status in Streptozotocin–Nicotinamide Induced Diabetic Rats. Evidence-Based Complementary and Alternative Medicine 2005; 2: 375–381.
24. Afshari L, Amani R, Soltani F, Haghighizadeh MH, Afsharmanesh MR. The relation between serum Vitamin D levels and body antioxidant status in ischemic stroke patients: A case–control study. Advanced Biomedical Research 2015; 4: 1-7.
25. Schettler V, Wieland E, Verwiebe R, Schuff-Werner P, Scheler F, Oellerich M. Plasma lipids are not oxidized during hemodialysis. Nephron 1994; 67: 42-47.
26. Roberta S, Luciane BP, Naiara S, Cinthia M, Roselia S, Jessié G, *et al*. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid

dehydratase in liver and kidney of streptozotocin-induced diabetic rats. Biochimie 2012; 94: 374-383.
27. Alok KD, Jhansee M, Deepak KD. *In vivo* Antioxidant Activity and Anti Hyperglycemic Relevant Enzyme Inhibition Properties of Petroleum Ether Extract of Traditionally Processed *Ocimum canum* Leaves. British Journal of Pharmaceutical Research 2014; 4: 1031-1040.
28. Sireesha K, Raghunandan N. Evaluation of *in vivo* antidiabetic and antioxidant activity of *Artocarpus hirsutus* seeds in streptozotocin-induced diabetic rats. Asian J Pharm Clin Res 2016; 9: 170-173.
29. Ramya R, Kalaiselvi M, Narmadha R, Gomathi D, Devaki K. Effect of *Alpinia calcarata* on glucose uptake in diabetic rats-an *in vitro* and *in vivo* model. Journal of Diabetes & Metabolic Disorders 2014; 13:1-11.
30. Uzma S, Bashir A, Mobasher A, Khalid H, Nadeem IB. Investigation of *in vivo* antioxidant activity of *Euphorbia helioscopia* latex and leaves methanol extract: a target against oxidative stress induced toxicity. Asian Pac J Trop Med 2014; 7: S369-S375.
31. Vijayaraj R, Sri Kumaran N. Evaluation of *in vivo* antidiabetic and antioxidant activity of *Achyranthes aspera* Linn. seeds by streptozotocin induced diabetic rats. International Journal of Green Pharmacy 2018; 12: 29-37.
32. Rajeswara Reddy S, Lavanya T, Narasimhulu G, Sathyavelu Reddy K. Regulation of cardiac oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats treated with aqueous extract of *Pimpinella tirupatiensis* tuberous root. Experimental and Toxicologic Pathology 2013; 65: 15–19.
33. Madhu CG, Ojha S, Bansal DD. Antioxidant status of streptozotocin diabetic rats. Ind J Exp Biol 1996; 34: 264 - 266.
34. Ali M, Hossein R, Zeinab S. Antioxidant Effect Of Pistachio Khinjuk On Alkali Injury Of Rabbit's Eye Cornea. Acta Medica Mediterranea 2016; 32: 1329-1333.
35. Subramani S, Leelavinothan P. Ameliorative effect of diosmin, a citrus flavonoid against streptozotocin nicotinamide generated oxidative stress induced diabetic rats. Chemico-Biological Interactions 2012; 195: 43–51.
36. Mohanasundaram S, D.Victor AD, Prasad Maddisetty PN. Hepato-renal protective effects of hydroethanolic extract of *Senna alata* on enzymatic and nonenzymatic antioxidant systems in streptozotocin induced diabetic rats. Integr Med Res 2016; 211: 1-8.

Cite this article as:

Benne Lakshmi Narsimhulu *et al*. Enzymatic and nonenzymatic antioxidant activities of *Cleome viscosa* and *Cleome gynandra* whole plant methanolic extracts in streptozotocin-induced diabetic rats. Int. Res. J. Pharm. 2018;9(6):118-124 http://dx.doi.org/10.7897/2230-8407.096101

Source of support: Nil, Conflict of interest: None Declared