Research Article

Centella asiatica Ameliorates Diabetic Complications and Oxidative Stress in Streptozotocin-Induced Diabetes Mellitus in Male Rats

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ABSTRACT

Effects of *Centella asiatica* upon oxidative stress and dysfunctions of liver, heart, kidney and testis in STZ-diabetic rats were investigated. Animals were divided into four groups Group 1, Control; Group 2, streptozotocin (STZ); Group 3, *C. asiatica* treated plus streptozotocin and Group 4, *C. asiatica*. Toxicological parameters including AST, ALT, ALP, LDH and CK-MB were significantly improved after treatment with *C. asiatica* (250 mg/kg BW, orally) in diabetic rats. Co-administration of *C. asiatica* ameliorated higher levels of plasma total cholesterol, triglycerides, total lipids and low density lipoprotein cholesterol (LDL-C) and increased the level of high density lipoprotein cholesterol (HDL-C). While, urea, creatinine and uric acid levels were decreased. A significant reduction of plasma Na^{+1}, K^{+1}, Mg^{+2} and Ca^{+2} that improved by *C. asiatica* in diabetic rats compared to non diabetics. *C. asiatica* treatment restored testosterone, FSH, LH, PRL and sperm count near to normal values in diabetic rats. In addition, SOD, CAT, GPX activities and GSH levels were restored by *C. asiatica* administration and consequently, the levels of lipid peroxidation were reduced in the selected organs as compared to diabetic animals. Also, treatment of rats *Centella asiatica* ameliorated DNA fragmentation. The protective effect of *C. asiatica* is mainly attributed to its antioxidant properties and pharmaceutical potent. These findings demonstrated the protective effect of *C. asiatica* on the antioxidant tissue defense system during streptozotocin-induced diabetes and its beneficial effect in preventing diabetic complications.

Keywords: *Centella asiatica*, diabetic complications, antioxidant enzymes, DNA fragmentation, rats.

INTRODUCTION

Diabetes is a prevalent systemic disease affecting a significant proportion of the population worldwide. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels[1]. Type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency in secretion and/or action of endogenous insulin. It has also been associated with an increased risk for developing atherosclerosis due to alteration in the blood lipid profile and raising the risk of cardiovascular diseases[2].

High reactivity of oxygen reactive species (ROS) determines chemical changes in virtually all cellular components, leading to lipid peroxidation. It has been suggested that enhanced production of free radicals and oxidative stress is the central event for the development of diabetic complications. Much attention has been focused on the key role of oxidative stress in the pathogenesis of different diabetic complications[3]. Antioxidant therapy is used as one of the most important treatment strategies for diabetic patients for the prevention and slowing of diabetic complications progression such as hyperlipidemia, hepatic damage[4].

Medicinal plants play a key role in human health care now-a-days. *Centella asiatica* (*Centella a.*) has a long history in the ancient Ayurvedic remedy, used in wound healing[5] and effective in treatment of stomach ulcer, hepatitis and asthma[6]. The plant is claimed to possess anticancer activity, anti-inflammatory[7] and antimicrobial[8]. Different parts of *C. asiatica* were found to contain high phenolic contents (quercetin, kaempherol, catechin, rutin, apigenin and naringin and volatile oils (caryophyllene, farnesol and elemene), which exhibit strong association with its antioxidative activities[9].

Diabetes mellitus poses a major health problem on both clinical and social plans also, for the onset of serious invaliding complications that frequently appear[10]. Accordingly, this study was designed to investigate the possible protective effects of *C. asiatica* on the diabetic complications and antioxidant status of hepatic, cardiac, renal and testicular tissues of STZ-diabetic rats, which are often prone to secondary complications of the disease.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma Chemical Co., Saint Louis, MO USA. *Centella asiatica*, Capsugel was purchased from Nature's Way Products, Inc. Springville, Utah 84663 USA. All other used chemicals in the experiment were of analytical grade.

Animal and experimental design

Adult male albino rats of *Wistar* strain weighing about 140–160 g were obtained from the animal house of the High Institute of Public Health, Alexandria University, Egypt. They were acclimatized to animal house conditions, fed on a standard chow diet and had free

access to water. The local committee approved the design of the experiments and the protocols were carried out according to the guidelines of the National Institutes of Health (NIH). After two weeks of acclimatization, twenty eight rats were divided into three groups as follows: The first group (7 rats) was used as a control, injected with the same volume of citrate buffer and received distilled water orally by gavage. The second group (14 rats) was injected intraperitoneally with a single dose of streptozotocin (STZ, 40 mg/kg BW) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). This group was divided into two subgroups (7 rats per each), the first subgroup was kept as diabetic and the second subgroup received *C. asiatics* suspended in distilled water (250 mg/kg BW/day), orally by gavage. The third group (7 rats) received *C. asiatics* suspended in distilled water (250 mg/kg body weight/day), orally by gavage for 28 days. Prior to administration of streptozotocin, the animals were fasted for 12h with free access to drinking water. STZ-injected animals exhibited massive glycosuria and hyperglycemia within 2-3 days. Blood was drawn from the tail vein and fasting blood glucose was estimated with a glucometer (Frankenberg, Germany). Rats were considered diabetic only if their fasting blood glucose levels exceeded 250 mg/dl[11].

Blood Collection and Tissue Preparation

At the end of experiment, rats were fasted for 12 hrs before being anesthetized and sacrificed by cervical dislocation. Blood samples were collected from the sacrificed animals and left in refrigerator for 30 min before centrifugation. The liver, heart, kidney and testis were quickly removed, washed in ice-cold, isotonic saline and blotted individually on ash free filter paper. The tissues were then homogenized in 0.1 M Tris–HCl buffer, pH 7.4 and the homogenate was centrifuged. The supernatant was used for the estimation of antioxidant enzymes (SOD, GPX, CAT) and other parameters (GSH and MDA).

Biochemical parameters

Liver function parameters were measured, namely: plasma aspartate transaminase (AST; E.C.2.6.1.1), alanine transaminase (ALT; E.C.2.6.1.2), alkaline phosphatase (ALP; E.C.3.1.3.1) and lactate dehydrogenase (LDH; E.C.1.1.1.27) activities were determined with kits from SENTINEL CH. (Via Principe Eugenio 5–20155 MILAN, Italy). Creatine kinase-MB (CK-MB) was estimated by the kinetic enzymatic method using commercial kits Laborlab®.

Also, total cholesterol (TC), triglycerides (TG), total lipids and high density lipoprotein (HDL-C) were determined with kits from SENTINEL CH. (Via Principe Eugenio 5-20155 MILAN-ITALY). Low density lipoprotein (LDL-C) was calculated.

Plasma urea and creatinine concentrations were analyzed by the method of Henry[12] and Patton & Crouch[13], respectively. Uric acid was determined by using kits from Biodiagnostic Co., Cairo, Egypt.

The concentration of some ions (Na^{+1}, K^{+1}, Mg^{+2}, and Ca^{+2}) in the plasma was determined by using a Perkin-Elmer 2380 atomic absorption spectrophotometer according to Eaton and Arnold[14].

Plasma concentrations of testosterone (Eiagen Testosterone kit, Italy), follicle stimulating hormone, luteinizing hormone, and prolactin (Erba Fertikit, Germany) were measured following an immunoenzymatic method with an ELISA reader, according to the standard protocol given in assay kit.

The level of reduced glutathione (GSH) was determined by the enzymatic method of Jollow[15]. The activity of superoxide dismutase (SOD; E.C.1.15.1.1) was estimated according to the method of Misra and Fridovich[16]. The activity of catalase (CAT; E.C.1.11.1.6) was determined by the method of Xu[17]. The activity of glutathione peroxidase (GPX; E.C.1.1.1.9) was measured by the method of Chiu[18]. Also, the level of MDA was measured by using the method of Ohkawa[19].

Sperm analysis

The testis and epididymis were sampled at the end of the experiment. Spermatozoa obtained from the cauda epididymis were subsequently diluted with physiological solution (20 µl) at 37 ^{o}C. The sample was located in the Makler chamber (Sefi-Medical Instruments, Germany). Analysis was realized using a CASA System – Supervision (Minitüb, Tiefenbach, Germany) with Olympus BX 51 (Olympus, Japan) microscope. The sperm count (million/ml), motile ratio (%) and abnormal sperm morphology (ASM, %) were evaluated in the experimental groups according to the method of Dunson[20]. A drop of 1% eosin-Y staining was added to the sperm sample on a microscope slide for morphological studies[21].

DNA fragmentation analysis

Genomic DNA was isolated from liver homogenate with the Qiamp DNA mini kit according to the manufacturer's instructions and electrophoresed on a 2% agarose gel stained with ethidium bromide[22]. The gel was then photographed under ultraviolet luminescence. In these conditions, damaged DNA appears as a ladder consisting of DNA fragments, whereas intact DNA is high molecular weight and does not migrate very far into the gel.

Statistical analysis:

All the data were statistically evaluated by SPSS/19 software (Chicago, IL, USA). Hypothesis testing methods included One Way Analysis of Variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All these results were expressed as Mean±S.E. for seven animals in each group.

RESULTS

Table (1) showed the activities of AST, ALT, ALP in plasma of control and STZ-diabetic rats. The activities of these enzymes were found to be significantly increased (P<0.05) in the plasma of diabetic rats. Oral administration of *C. asiatica* resulted in the near normalization of these enzyme activities in the plasma of diabetic rats compared to untreated diabetic rats. Also, Table 1 showed a marked elevation in the activities of CK-MB and LDH enzymes in the plasma of diabetic rats. Co-treatment with *C. asiatica* resulted in significant reduction in the levels of these enzymes towards near normal values as compared to diabetic rats.

The rats in group 2 showed a marked increase in plasma total cholesterol (TC), triglycerides (TG), total lipid, low density lipoprotein (LDL-C) while, a significant reduction in HDL-C level when compared to control group. Oral administration of *C. asiatica* partially improved the changes in lipid profile in the diabetic group as compared to diabetic group (Table 2). It was also noticed that the *C. asiatica* administration completely improved HDL levels in diabetic rats.

In table (3), urea, creatinine and uric acid levels were significantly elevated in DM rats (P<0.05) when compared to control animals. Co-administration of *C. asiatica* significantly lowered urea, creatinine and uric acid levels in STZ-diabetic rats compared to the untreated diabetic group. Also, administration of *C. asiatica* restored the concentration of Na^{+1}, K^{+1}, Mg^{+2} and Ca^{+2} to normal levels as compared to diabetic group.

In table 4, the level of testosterone was significantly decreased (P<0.05) while, the levels of circulating follicle stimulating hormone, luteinizing hormone and prolactin were significantly increased in STZ-treated group. The results clearly showed that treatment with *C. asiatica* partially improved the levels of testosterone, FSH, LH and prolactin. In addition, the administration of STZ decreased the sperm count, motility and increased the sperm abnormalities (Table 4 & Figure 1). *C. asiatica* treatment partially ameliorated the reduction in the number of sperms and reduced the abnormalities of the sperms as well as, elevated their motility as shown in groups 3 and 4.

The GSH level was significantly (P<0.05) decreased in contrast, the level of MDA was significantly increased in diabetic rats in comparison to the control group. The *C. asiatica* treatment partially improved these alterations while, it restored the MDA level to near normal value specially the heart tissue. The mean activities of SOD, CAT and GPx were significantly (P<0.05) decreased in the diabetic rats compared to the control group (Tables 5 & 6). Co-treatment with *C. asiatica* (250 mg/kg BW/ day, orally) resulted in a significant enhancement in the levels of these enzymes as compared to diabetic group but did not reach to the normal values.

Also, it was noticed that *C. asiatica* administration (250 mg/kg BW/day, orally) alone had no effect on the measured parameters. On the other hand, it showed a significant increase in the GSH levels and the activities of GPx, SOD, CAT and kept MDA level less than the control.

Genotoxicity studies

To explore the mode of cell death, the present study has done DNA gel electrophoresis to recognize the nature of cellular death (necrosis and/or apoptosis) in the liver. DNA isolated from STZ-exposed rats showed a mainly ladder (a hallmark of apoptosis) on the agarose gel (Figure 2) compared to control. Exposure to STZ elicited the hepatic DNA damages. While, treatment of rats with 250 mg/kg BW/day *Centella asiatica* reduced DNA fragmentation.

Table 1: Effect of *Centella asiatica* on liver and heart functions in STZ-induced diabetic rats.

Groups Parameters	Control	Diabetic	Diabetic + Centella asiatica	Centella asiatica
AST (U/L)	47.20±2.2731	265.00±1.3317^{a}	97.60±1.2572^{ab}	47.06±1.3462^{b}
ALT (U/L)	38.25±0.4425	175.50±0.5481^{a}	60.00±2.2367^{ab}	38.00±1.3562^{b}
ALP (U/L)	47.00±2.7552	145.00±1.1405^{a}	67.00±3.5472^{ab}	48.16±2.3472^{b}
CK-MB (U/L)	84.00±1.9235	203.00±4.2544^{a}	176.80±9.5363^{ab}	80.40±3.0430^{b}
LDH (U/L)	182.60±6.0712	390.60±8.3880^{a}	209.40±6.1855^{ab}	175.40±4.2261^{b}

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I. - b, Comparison of Group III, Group IV vs Group II.

Table 2: Effect of *Centella asiatica* on lipid profile in STZ-induced diabetic rats.

Groups Parameters	Control	Diabetic	Diabetic + *Centella asiatica*	*Centella asiatica*
Cholesterol (mg/dl)	81.10±2.8530	490.00±9.7652[a]	297.20±6.8073[ab]	82.80±5.3141[b]
Triglycerides (mg/dl)	37.80±1.7146	434.30±5.5642[a]	295.00±5.0000[ab]	38.60±10.5953[b]
Total Lipids (mg/dl)	297.20±9.0741	627.00±6.9269[a]	320.70±8.0212[ab]	219.70±1.3643[ab]
LDL-C (mg/dl)	44.00±3.1780	388.00±6.1514[a]	173.00±3.1937[ab]	33.6±4.1904[ab]
HDL-C (mg/dl)	40.40±1.3928	23.00±1.7204[a]	45.20±2.5951[b]	41.40±1.2885[b]

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I. - b, Comparison of Group III, Group IV vs Group II.

Table 3: Effect of *Centella asiatica* on urea, creatinine, uric acid, Na^{+1}, K^{+1}, Mg^{+2} and Ca^{+2} levels in STZ-induced diabetic rats.

Groups Parameters	Control	Diabetic	Diabetic + *Centella asiatica*	*Centella asiatica*
Urea (mg/dl)	30.60±1.0296	73.00±3.3615[a]	35.8±2.0833[b]	30.40±1.1402[b]
Creatinine (mg/dl)	0.26±0.3839	1.68±0.1147[a]	0.33±0.1393[b]	0.27±0.0361[b]
Uric acid (mg/dl)	1.34±0.2000	2.26±0.1600[a]	1.48±0.1077[b]	0.96±0.2350[b]
Na^{+1} (ppm)	139.30±1.9849	93.30±1.9340[a]	129.20±4.7256[b]	139.80±1.7720[b]
K^{+1} (ppm)	4.24±0.2461	2.10±0.1077[a]	3.85±0.1382[b]	4.10±0.1304[b]
Mg^{+2} (ppm)	2.15±0.1203	1.30±0.1273[a]	2.45±0.2083[b]	2.24±0.1029[b]
Ca^{+2} (ppm)	9.28±0.1855	8.00±0.1378[a]	9.00±0.1360[b]	9.08±0.1828[b]

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I. - b, Comparison of Group III, Group IV vs Group II.

Table 4: Effect of *Centella asiatica* on sex hormones, sperm count, abnormality % and motility % in STZ-induced diabetic rats.

Groups Parameters	Control	Diabetic	Diabetic+ *Centella asiatica*	*Centella asiatica*
Testosterone (pg/m l)	7.50±0.3435	2.78±0.1463[a]	3.60±0.2345[ab]	7.22±0.3146[b]
FSH (ng/ml)	4.32±0.2782	10.34±0.3156[a]	6.78±0.2871[ab]	4.72±0.1393[b]
LH (ng/ml)	1.83±0.1393	4.49±0.1208[a]	3.18±0.2817[ab]	1.98±0.1934[b]
PRL (ng/ml)	0.46±0.9274	7.30±1.2558[a]	3.11±0.5843[ab]	0.50±0.1581[b]
Sperm Count (million/ml)	59.20±3.3226	29.40±1.3267[a]	40.60±1.5684[ab]	60.00±2.4207[b]
Abnormality (%)	7.00±0.8609	30.20±1.7720[a]	17.00±1.8708[ab]	6.80±0.5219[b]
Motility (%)	70.00±1.7205	12.10±0.9987[a]	48.00±1.0296[ab]	80.00±3.5581[ab]

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I. - b, Comparison of Group III, Group IV vs Group II.

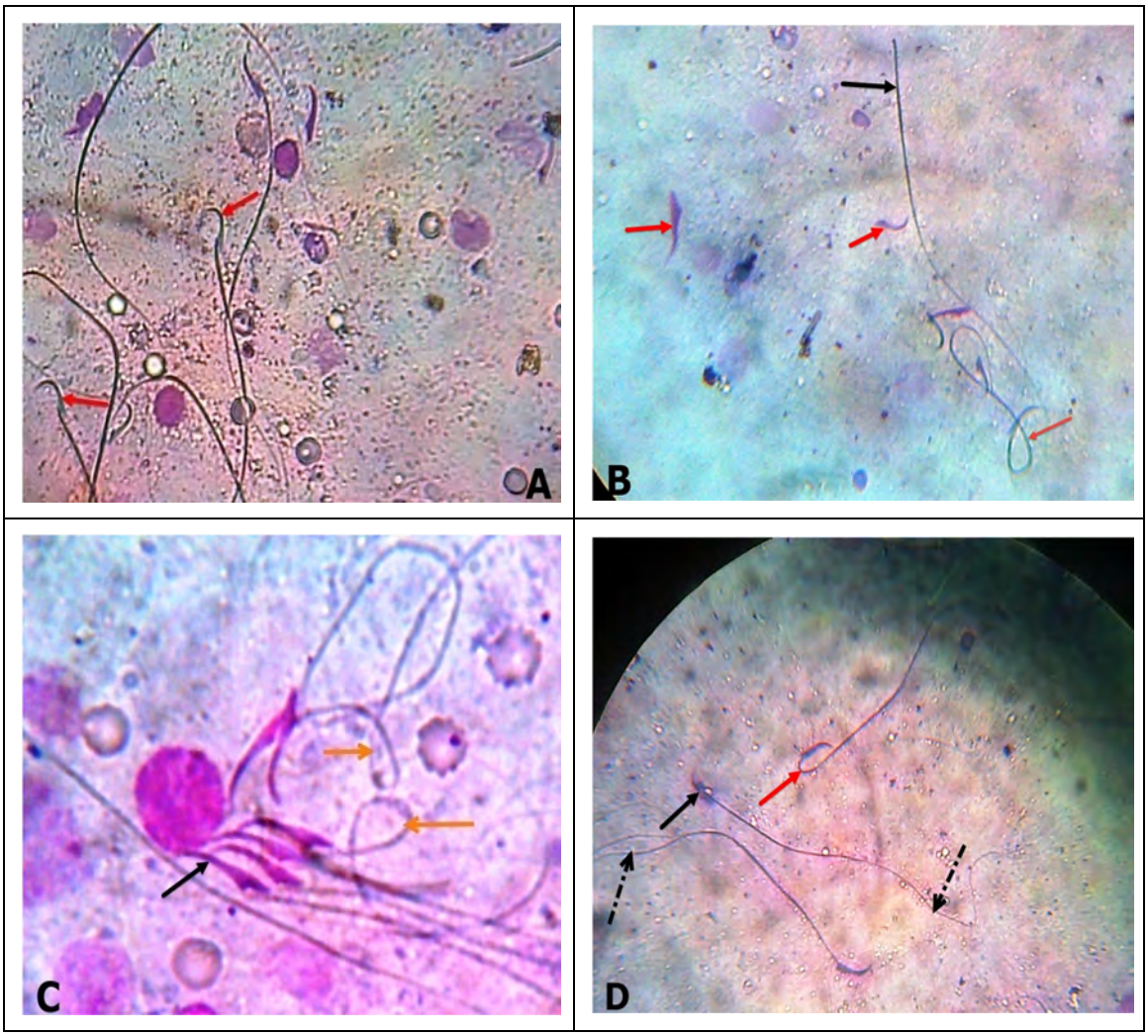

Figure 1: Photomicrograph of normal rat Sperms from control group (A); normal hooked head, normal neck and normal tail. Photomicrographs of abnormal rats sperms from the diabetic (STZ)-treated group (B); head without tail (red arrow), pin point shaped sperm (black arrow), coiled tail (orange arrow) (C); sperms aggregation (black arrows), coiled tail (orange arrows). (D); bent neck (red arrow), hookless (black arrow) and long folded tail (dotted arrow) (EosinY stain, 400 x).

Table 5: Effect of *Centella asiatica* on the level of GSH & MDA and the activities of antioxidant enzymes of liver and heart in STZ-induced diabetic rats.

Groups	Control		Diabetic		Diabetic + *Centella asiatica*		*Centella asiatica*	
Parameters	Liver	Heart	Liver	Heart	Liver	Heart	Liver	Heart
GSH (U/g tissue)	60.20 ±2.4779	64.00 ±1.5811	16.30 ±1.3379^{a}	16.80 ±0.8602^{a}	41.60 ±2.5613^{ab}	29.20 ±1.2409^{ab}	62.40 ±4.3658^{b}	67.40 ±4.0199^{b}
MDA (nmol/g tissue)	19.00 ±0.7071	23.00 ±1.7321	68.52 ±1.1576^{a}	52.62 ±3.1401^{a}	41.10 ±3.1321^{ab}	24.29 ±2.5417^{ab}	18.20 ±1.6852^{b}	18.80 ±2.1772^{ab}
SOD (U/mg protein)	81.22 ±1.2806	71.60 ±0.9274	25.31 ±1.5621^{a}	26.00 ±1.9493^{a}	39.90 ±2.7946^{ab}	36.00 ±2.1679^{ab}	80.41 ±1.5033^{b}	79.41 ±1.0770^{b}
CAT (U/mg protein)	42.80 ±1.4967	50.40 ±1.8056	10.64 ±0.6633^{a}	12.63 ±0.9274^{a}	21.30 ±1.2409^{ab}	28.82 ±2.8178^{ab}	44.00 ±2.0248^{b}	55.39 ±3.0757^{b}
GPx (U/mg protein)	68.20 ±1.0198	61.61 ±3.9825	15.60 ±1.2083^{a}	16.05 ±1.6612^{a}	37.10 ±1.8868^{ab}	36.35 ±1.4353^{ab}	69.90 ±2.1932^{b}	67.00 ±3.6055^{b}

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I.

- b, Comparison of Group III, Group IV vs Group II.

Table 6: Effect of *Centella asiatica* on the level of GSH & MDA and the activities of antioxidant enzymes of kidney and testis in STZ-induced diabetic rats.

Groups	Control		Diabetic		Diabetic + *Centella asiatica*		*Centella asiatica*	
Parameters	Kidney	Testis	Kidney	Testis	Kidney	Testis	Kidney	Testis
GSH (U/g tissue)	70.40 ±1.2207	70.20 ±1.2409	29.80 ±1.3565 [a]	11.20 ±0.8602[a]	48.00 ±2.6058[ab]	24.00 ±1.9236[ab]	69.80 ±0.8622[b]	74.10 ±3.0561[b]
MDA (nmol/gtissue)	12.70 ±0.6633	13.20 ±1.0677	51.00 ±1.4142[a]	68.70 ±2.2672[a]	28.90 ±1.4525[ab]	42.20 ±2.7092[ab]	13.50 ±1.1576[b]	14.00 ±1.4142[b]
SOD (U/mgprotein)	71.70 ±2.7368	72.60 ±4.7916	19.32 ±0.8616[a]	14.02 ±0.8616[a]	47.30 ±3.0800[ab]	28.40 ±2.5807[ab]	78.01 ±2.8636[ab]	70.80 ±4.1881[b]
CAT (U/mgprotein)	61.30 ±2.1541	45.60 ±2.0396	22.40 ±1.7205[a]	14.30 ±2.3108[a]	29.80 ±1.3929[ab]	29.80 ±1.3929[ab]	58.60 ±2.9766[b]	50.30 ±1.1576[b]
GPx (U/mgprotein)	59.00 ±0.7071	76.00 ±2.2226	32.80 ±1.8547[a]	21.00 ±1.5814[a]	51.30 ±1.0770[ab]	41.00 ±1.6125[ab]	67.40 ±1.0773[ab]	72.40 ±4.1665[b]

Values are given as: Mean ± S.E. of seven animals in each group; - a, Comparison of Group II, Group III and Group IV vs Group I. - b, Comparison of Group III, Group IV vs Group II.

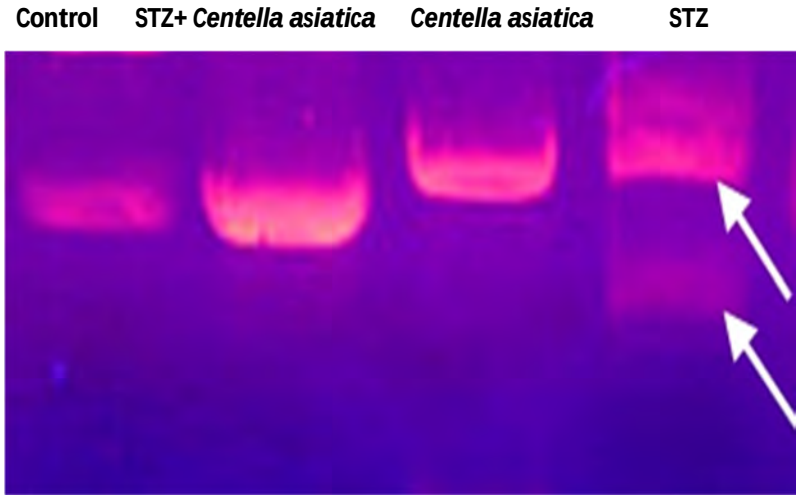

Figure 2: DNA fragmentation pattern on agarose gel. DNA isolated from liver: control lane 1, STZ+ *Centella asiatica*-treated rats lane 2, *Centella asiatica*- treated rats lane 3 and STZ- treated rats lane 4, respectively. Arrows indicate ladder formation. *Centella asiatica* reduced all the STZ-induced pro-apoptotic events in hepatic tissue.

DISCUSSION

Liver enzymes such as ALT, AST, and ALP reflect the different functions of the liver, such as hepatocellular integration, formation, and subsequent free flow of bile and protein synthesis. ALT and AST are important indicators of hepatocellular damage[23]. In the present study, oral administration of *C. asiatica* to diabetic rats alleviated the enhancement in the activities of ALT, AST and ALP. This means that *C. asiatica* has some hepatoprotective potentials in normal and diabetic rats by decreasing plasma ALT, AST and ALP levels. *C. asiatica* is a rich source of bioactive compounds and is used as a folk medicine for the treatment of various diseases, including diabetes. Medicinal plant and natural antioxidant with hepatoprotective action could prevent or be helpful in reducing the complications of hepatic damage seen in diabetic patients[24,25].

In the same line with the findings of Jagdish & Shah[26] CK-MB and LDH activities of untreated STZ-diabetic rats were significantly elevated indicating damage to cardiac muscle. Wiernsperger[27] stated that the quantity of CK-MB and LDH released is a measure of necrosis in the heart during diabetes. The most common macrovascular complication of diabetes is atherosclerosis, which increases the risk of myocardial infarction, stroke and peripheral artery disease[28]. The present work stated that co-treatment with *C. asiatica* (250 mg/kg BW/day) effectively counteracted the alterations in these enzymes. The active ingredient triterpenes (acetic acid, asiaticoside) may be responsible for the cardioprotective effect of *C. asiatica*. It has been reported that another structurally related triterpene (arjunolic acid) possess the cardio-protective effect by inhibition of enzymatic activity against isoproterenol induced myocardial damage[29]. The

present results was consistent with the results of Gnanapragasam[30] and Pragada[31].

The most commonly observed lipid abnormalities in diabetes were hypercholesterolemia and hypertriglyceridemia and contribute to coronary artery disease[32,33]. In fact, lipid abnormalities accompanying with atherosclerosis are the major cause of cardiovascular disease in diabetes. These results were in accordance with the results of Ravi[34]. In insulin-resistant and T2DM, high fluxes of triglycerides exist because of unsurpassed lipolysis in the adipose tissues, as well as hepatic overproduction of triglyceride-rich particles. This excessive lipid exposure, in the presence of impaired glucose utilization, results in accumulation of triglycerides in non-adipose tissues, including the myocardium[35]. Soliman[36] found that diabetic rats exhibited an increase in the levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and a decrease in the level of high density lipoprotein cholesterol (HDL-C).

The lipid profile alterations in diabetic rats were reduced after *C. asiatica* administration. Treatments of the diabetic rats with *Centella* extracts showed a significant decrease in blood glucose and increase in insulin level and improved lipid profile as cholesterol, TG, LDL-C and HDL-C[37,38]. The mechanism of hypolipedemic and antiatherogenic action of medicinal plant may be due to the inhibition of dietary lipid absorption in the intestine or its production by the liver, stimulation of the biliary secretion of cholesterol and cholesterol excretion in the feces[39]. The protective action of *Centella* extracts may also be due to the inhibition of enzymes and proteins that involve lipid and lipoprotein metabolism[40].

Diabetic nephropathy is one of the most common and most devastating complications of diabetes. It includes hyperfiltration and renal hypertrophy[41]. The observed increase in plasma levels of urea, creatinine and uric acid might be due to STZ-induced metabolic disturbances as well as renal dysfunction. The levels of non-protein nitrogenous substances are always used as significant markers for the assessment renal dysfunction characterized by proteinuria. Also, the elevation of these parameters may be attributed to protein catabolism and glomerular injury[42]. *C. asiatica* treated rats significantly decreased urea, creatinine and uric acid levels indicating its potent antioxidant property. This was in agreement with Zheng[43] who demonstrated that, asiaticoside has a protective effect against acute kidney injury.

The present study reported that the mineral status in diabetic rats was reduction in sodium, potassium, magnesium and calcium concentrations. The loss of these minerals might be attributed to impaired absorption and/or the excess excretion of these minerals in urine (glycosuria), which may induce a deficiency or marginal level in the blood[44,45].

Male infertility is a common threat nowadays and it has increased rapidly because of hyperglycemia[46,47]. STZ disturbed the hormonal levels, the sperm count, motility and increased the sperm abnormalities. In the present study, oral administration of *C. asiatica* to diabetic rats improved the disturbances in the hormonal levels and improved semen analysis. The bark and leaves of the plant were found to enhance sexual potency in men. They have also been found to promote testosterone production[48,49]. The seeds also contain flavonoids and steroids which are associated with functions related to fertility enhancement[50,51].

In case of diabetes, persistent and chronic hyperglycaemia may generate free radicals and reactive oxygen species (ROS), which trigger an oxidative stress. Researches reported the role of oxidative stress as a central factor in the onset and progression of diabetic complications such as hyperlipidemia and hepatic damage[52,53].

Several studies have been reported that antioxidant enzyme activities are reduced in diabetes[54,55]. They added that antioxidant enzymes are important in mitigating free radical induced cell injury.

The production of lipid peroxides was significantly decreased by *C. asiatica* treatment. This result may be due to the active components of *C. asiatica* such as disulphides and their oxidized thiols which has been reported to have an antioxidative effect as reported by Bian[56].

They postulated that the *C. asiatica* had a suppressive effect on myloperoxidase activity, which in turn may decrease the production of oxygen species and reduce concomitant tissue damage.

Pittella[57] found that aqueous extract *C. asiatica* showed to counteract lead-induced oxidative stress in male rats. Hashim[58] reported that antioxidants in *Centella* (84%) are comparable to vitamin C (88%) and grape seed extract (83%). These findings were in agreement with the observation of Ramachandran[59] who stated that asiatic acid (AA), a triterpenoid derivative of *Centella asiatica*, has shown significant biological effects of antioxidant and anti-inflammatory activities. *Centella asiatica* showed a significant cardioprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation as well as elevated the levels of antioxidant enzymes[60].

Like other macromolecules such as lipids, proteins and nucleic acids are also attacked by free radicals to cause oxidative DNA damage. In the present study, STZ had degraded the DNA of liver tissue by generating free radicals. On the other hand, co-treatment of *Centella asiatica* appreciably reduced the DNA fragmentation which indicated by DNA ladder banding pattern. Similar results were reported by Joy and Nair who reported that gotu kola has been found to significantly reduce damage to DNA[61].

Therefore, antioxidants play significant role in partly preventing DNA damage. Various phenolic antioxidants

such as flavonoids, tannins and coumarins have been shown to scavenge radicals and thus partly prevent the DNA damage[62].

CONCLUSION

It can be concluded that the protective efficacy of *Centella asiatica* may be due to the presence of a high content of flavonoids, which acts synergistically as antioxidants and has beneficial effects in reducing functional disturbances and oxidative stress in streptozotocin-induced diabetes mellitus in male rats. From the present findings, it was well documented that the *C. asiatica* played a part in the management of diabetes and slowing or prevention of its complications.

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