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THE HYPOGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *CENTELLA ASIATICA* AGAINST STZ-INDUCED DIABETIC DISORDERS IN RATS

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ABSTRACT

The effect of *Centella asiatica* on diabetes markers, glucose metabolizing enzymes and pancreas antioxidants was investigated. *C. asiatica* showed a significant antidiabetic activity by reducing the elevated glucose and glycosylated hemoglobin ratio. The insulin, insulin index, C-peptide levels, the liver and muscle glycogen and body weights were improved in the treated diabetic rats. *C. asiatica* could normalize the activities of the glucose metabolizing enzymes and improved the decrease in the red and white blood cells, hemoglobin, haematocrit and platelets. The pancreatic antioxidants were also increased by *C. Asiatica* treatment, while, malondialdehyde was decreased. The results showed that diabetes produced a significant decrease in DNA, RNA and RNA/DNA ratio that was normalized after *C. asiatica*. The histopathological studies showed regeneration of the pancreatic cells after treatment of the diabetic rats with *C. asiatica*. In conclusion, *C. asiatica* might be useful for diabetes management and could be able to antagonize diabetic disorders.

KEYWORDS: ***Centella asiatica*, diabetes, carbohydrate metabolism, hematology, rats.**

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INTRODUCTION

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world. Diabetes mellitus results in hyperglycemia and is characterized as type 1 in absolute insulin deficiency or type 2 in insulin resistance due to receptor insensitivity to endogenous insulin (1). As per WHO, 346 million people worldwide have diabetes and it is also projected that death due to this will be the double between 2005 and 2030 (2). The beneficial effect of synthetic drugs provides good glycemic control, but long term use have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (3). Herbs have been used as human food and medicines. Traditional medicinal plants are widely used in folk medicine to treat many diseases (4). There is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (5). *Centella asiatica* (Gotu Kola) is a widely used traditional remedy in both Africa and India. *C. asiatica* (L.) urban is a herb of the family *Umbelliferare*. It has been used widely in folk medicine to treat a wide range of illness. It has antipyretic (6) and anti-inflammatory effects (7) on different animal models. It is also, useful in vascular diseases such as venous hypertension and atherosclerosis (8). The most major components of *C. asiatica* have been divided in to major collections, these groups involved triterpenoids, saponins and their aglycones correspondents (asiaticoside, madecassoside, asiatic and madecassic acids), pectin, volatile oil, traces of alkaloids, and others (9,10). Also, different parts of *C.asiatica* were found to contain high phenolic contents (quercetin, kaempherol, catechin, rutin, apigenin and naringin and volatile oils such as caryophyllene, farnesol and elemene), which exhibit strong association with its antioxidative activities (11). The plant also contains amino acids, *viz*. aspartic acid, glycine, glutamic acid, *α*-alanine and phenylalanine have also been documented in the plant (12). It is also rich in vitamin C, vitamin B1, vitamin B_2, niacin, carotene and vitamin A. The total ash contains chloride, sulphate, phosphate, iron, calcium, magnesium, sodium and potassium (13). So, the present study was undertaken to investigate: (1) the effectiveness of C. asiatica in reducing the hyperglycemia, diabetic disorders and oxidative stress in the STZ-induced diabetic rats. (2) the role of C. asiatica in alleviating the hematological and some biochemical alterations in STZ-induced diabetic rats. The outcome would provide important information about its usefulness as a therapeutic agent.

MATERIALS AND METHODS

(i) 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.

(ii) 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 body weight) 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 body weight/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 fourteen 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 **(**14). The body weight was determined after the 1st and 2nd week. During the experimental period blood glucose levels were determined at regular intervals. Insulin index was calculated by Quantitative Insulin Sensitivity Check Index (QUICKI) method.

(iii) 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. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 860 × g for 20 min and stored at –60 ˚C. Liver, muscle and pancreas were immediately removed and washed using the chilled saline solution. These tissues were minced and separately homogenized (10% w/v) using a Homogenizer (Potter-Elvehjem) in ice-cold sodium, potassium phosphate buffer (0.01M, pH 7.4) containing 1.15% of KCl. The homogenates were centrifuged at 10,000 ×g for 20 min at 4□C and the supernatant was used for assaying of the enzyme activities and biochemical parameters.

(iv) Biochemical parameters

Stored plasma samples were analyzed for glucose, insulin, c-peptide and the activities of glucokinase (GK; EC 2.7.1.2) and the α-amylase (EC 3.2.1.1) were assayed using kits from (Sigma Chemical Co., Saint Louis, MO USA). The liver and muscle glycogen contents were determined using the anthrone method as described previously by Chau et al. (15). Pancreas lipid peroxidation end product, MDA, was measured as thiobarbituric acid reactive substance (TBARS). Also, the levels of GSH and the activities of the antioxidant enzymes including superoxide dismutase (SOD; EC.1.15.1.1), the catalase enzyme (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC.1.1.1.9) were assayed using commercial assay kits according to the manufacturer's instructions.

(v) Hematological parameters

Anticoagulated blood was tested, shortly after collection, for the hematological parameters including red blood cells (RBC's), hemoglobin content (Hb) haematocrit value (Hct), white blood cell (WBC's) and platelet (PLt) were estimated by Particle Counter (ERMA Inc., Tokyo. Model PCE-210). Glycosylated hemoglobin (HbA1c) was estimated by enzyme linked immunosorbent assay (ELISA) kits (Boehringer Mannheim, Germany).In a pancreatic DNA analysis (Diphenylamine assay), 2.0 ml of the whole homogenate (WH) was measured and 2.0 ml of the diphenylamine reagent (Dissolved 0.7g of diphenylamine in 50 ml of glacial acetic acid and 0.75 ml of conc. H_2So_4 was added. Just prior to use, 0.25 ml of cold 1.6 % acetaldehyde was added (Prepared in a fume hoods) the tubes were allowed to cool to room temperature and the observances were read at 600 nm against a reaction blank. In RNA analysis (Orcinol assay), 0.5ml of the (WH) was measured and made up to 2.0 ml with 5% trichloroacetic acid (TCA). Then 2.0 ml of orcinol reagent (Dissolved 0.5 g of orcinol in 50 ml of 0.1% Fe cl_3 in conc. HCl (prepared in a fume hood) was added and mixed. The orcinol reagent was prepared just prior to use. The tubes were heated in a boiling water bath for 15 minutes. The tubes were removed and allowed to cool to room temperature and the absorbances were read at 640 nm against a reaction blank in a spectrophotometer.

(vi) Histopathological examination of pancreas

Histopathological examination was carried out according to Drury et al. (1980). The excised pancreas specimens were isolated and immediately fixed in 10% formalin, then treated with conventional grade of alcohol and xylol and then paraffin embedded. Paraffin blocks were sectioned into 4-5 µm thick sections. The sections were stained with Haematoxylin and Eosin (H&E) stain for studying the histopathological changes.

(vii) Statistical methods

The results were analyzed using the SPSS computer software package version 16.0 (Chicago, IL, USA). Data were presented as mean ± SE. Data were evaluated by one-way ANOVA followed by post hoc the Duncan multiple test. Values were considered statistically significant at P<0.05.

RESULTS

Diabetes induced by STZ resulted in a significant (p<0.05) increase in the level of plasma glucose, glycosylated hemoglobin (HbA1c) and a significant (p<0.05) decrease in the level of plasma insulin and C-peptide in diabetic rats compared to normal control rats. Oral administration of C. *asiatica* (250 mg/kg body weight), daily for a period of 14 days to diabetic rats, significantly (P<0.05) decreased the level of plasma glucose and glycosylated hemoglobin (HbA1c) and significantly (P<0.05) increased the level of plasma insulin and C-peptide compared to diabetic rats (Table 1). Also, in Table 1, all diabetic rats had QUICKI less than 0.31, with subject groups having mean QUICKI values of 0.317, 0.311 and 0.316 for control, treated diabetics and *C. asiatica* treated rats, respectively.

Table 1

Effect of Centella asiatica on plasma glucose level, insulin, C-peptide, and glycosylated haemoglobin (HbA1c) in STZ- induced diabetic rats.

Group Parameter	Control (Group I)	Diabetic (Group II)	Diabetic + *Centella asiatica* (Group III)	*Centella asiatica* (Group IV)
Plasma glucose level (mg/dL)	94.80 ±1.2409	560.00 ±1.5165 [a]	123.80 ±3.2155 [b]	89.00 ±0.707 [b,c]
Insulin (mlu/ml)	14.96 ±0.4456	5.86 ±0.4365 [a]	13.08 ±0.7644 [b]	16.28 ±0.716 [b]
C-peptide (ng/ml)	1.370 ±0.1384	0.481 ±0.0130 [a]	1.023 ±0.0514 [b]	1.420 ±0.0051 [b,c]
Glycosylated Hemoglobin (HbA1c)%	5.02 ±0.03602	14.12 ±0.3325 [a]	8.54 ±0.5202 [b]	5.22 ±0.3308 [b,c]
Insulin index (QUICKI values)	0.317 ±0.0011	0.284 ±0.0026 [a]	0.311 ±0.0024 [b]	0.316 ±0.0020 [b,c]

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

Data in Table3 indicated that treatment with streptozotocin caused a significant (P<0.05) reduction in the levels of α-amylase, glucokinase, liver and muscle glycogen. Oral administration of *C. asiatica* (250 mg/kg) to diabetic rats significantly increased α-amylase, glucokinase, liver and muscle glycogen and restored the marker enzymes to near control levels.

Table 2

Effect of Centella asiatica on α-amylase, glucokinase, liver and muscle glycogen in STZ- induced diabetic rats.

Group Parameter	Control (Group I)	Diabetic (Group II)	Diabetic + *Centella asiatica* (Group III)	*Centella asiatica* (Group IV)
α-amylase (U/L)	246.83 ±1.8000	133.80 ±3.2619 [a]	198.62 ±3.3592 [b]	240.04 ±1.4005 [b,c]
Glucokinase (U/L)	384.00 ±19.3623	196.00 ±12.2623 [a]	312.00 ±21.9763 [a,b]	373.00 ±39.5785 [a,b]
Liver Glycogen (mg/g tissue)	2.69 ± 0.1250	1.43 ±0.1281 [a]	2.49 ±0.0312 [b]	2.66 ±0.1364 [b]
Muscle Glycogen (mg/g tissue)	0.311 ±0.0241	0.201 ±0.0130 [a]	0.299 ±0.0422 [b]	0.315 ±0.0351 [b]

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

Results in Table 3 indicated that treatment with streptozotocin caused a significant (P<0.05) elevation in the level of MDA combined with inhibition in the activities of the antioxidant enzymes (SOD, CAT and GPx) in the pancreas of diabetic rats. Meanwhile, the changes in the activities of these enzymes were confirmed the histologically damage shown in the pancreas (Figure 2). This disturbance was also manifested by significantly decreased in the level of pancreas GSH (P<0.05). On the other hand, co-administrations of *C. asiatica* with streptozotocin alleviated its harmful effects on the levels of GSH and MDA. In addition, it increased the activities of SOD, CAT and GPx enzymes (Table 3). The improved levels of these parameters were observed likely to near normal values of the control group.

Table 3
Effect of Centella asiatica on the specific activity of pancreatic antioxidant enzymes in STZ- induced diabetic rats.

Group / Parameter	Control (Group I)	Diabetic (Group II)	Diabetic + *Centella asiatica* (Group III)	*Centella asiatica* (Group IV)
GSH (mg/g tissue)	80.80 ±1.6553	28.00 ±1.0000 [a]	51.00 ±2.3022 [a,b]	76.50 ±2.0639 [b]
SOD (u/mg protein)	72.40 ±3.4728	40.30 ±2.2716 [a]	60.50 ±0.9273 [a,b]	78.01 ±1.5166 [b,c]
CAT (u/mg protein)	46.40 ±2.5807	22.43 ±1.0296 [a]	38.42 ±1.8055 [a,b]	45.20 ±3.2156 [b]
GPx (u/mg protein)	61.20 ±2.0347	28.00 ±1.3038 [a]	42.40 ±1.3638 [a,b]	60.80 ±2.8178 [b]
MDA (n.mol/gmtissue)	17.60 ±1.2083	43.80 ±2.1307 [a]	23.20 ±1.4283 [a,b]	16.80 ±1.5937 [b]

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

The significant decrease in the levels of RBC's, Hb, Hct, WBC's and PLt observed in the diabetic animals was drastically increased to near normal level after the administration of *C. asiatica* at the dose of 250 mg/kg body weight/day (Table 4).

Table 4
Effect of Centella asiatica on hematological parameters in STZ- induced diabetic rats.

Group / Parameter	Control (Group I)	Diabetic (Group II)	Diabetic + *Centella asiatica* (Group III)	*Centella asiatica* (Group IV)
RBC (10^6/mm^3)	5.000 ±0.1140	3.3400 ±0.1077[a]	4.3400 ±0.0510[b]	5.000 ±0.0316[b]
Hct (%)	49.200 ±0.7349	30.060 ±0.8316[a]	41.140 ±0.4238[b]	49.000 ±0.7071[b]
Hb (g/dl)	14.800 ±0.2000	9.210 ±0.3100[a]	12.070 ±0.1044[b]	14.660 ±0.2993[b]
PLt (10^3/mm^3)	296.800 ±3.8393	176.600 ±1.7764[a]	275.400 ±4.2615[b]	304.000 ±2.2136[b]
WBC (10^3/mm^3)	12.000 ±3.7416	5.060 ±1.6309 [a]	8.660 ±2.3790 [b]	12.780 ±4.5650[b]

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

Table 5 presents the results of the effects of the *C. Asiatica* treatment of DNA, RNA and RNA/DNA ratio levels in *Wistar* albino rats. The results showed that diabetes independently produced a significant decrease (P<0.05) in the values of DNA, RNA and RNA/DNA ratio compared to control. However, co-administration of *C. asiatica* produced a significant increase (P<0.05) in values of DNA, RNA and RNA/ DNA ratio relative to control.

Table 5
Effect of Centella asiatica on DNA, RNA and RNA/DNA of pancreas in STZ- induced diabetic rats.

Parameters Groups	DNA mg/g tissue	RNA mg/g tissue	RNA/DNA ratio
Control (Group I)	56.20 ±1.06771	186.60 ±1.15758	3.328 ±0.04663
Diabetic (Group II)	25.40 ±0.92736 [a]	106.00 ±3.08221 [a]	4.170 ±0.09351
Diabetic + *Centella asiatica* (Group III)	37.00 ±0.70711 [a,b,c]	157.20 ±1.24097 [a,b,c]	3.172 ±0.08411 [b,c]
Centella asiatica (Group IV)	53.00 ±0.70711 [b,c]	178.20 ±6.28013 [b,c]	3.303 ±0.05543 [b,c]

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

Figure. 1 showed the changes in the body weight of the experimental groups. The mean body weight of diabetic rats decreased significantly (P<0.05) compared to all other groups. After treatment with *C. asiatica*, there were a significant (p<0.05) increase in body weight of group III and group IV when compared with the control group.

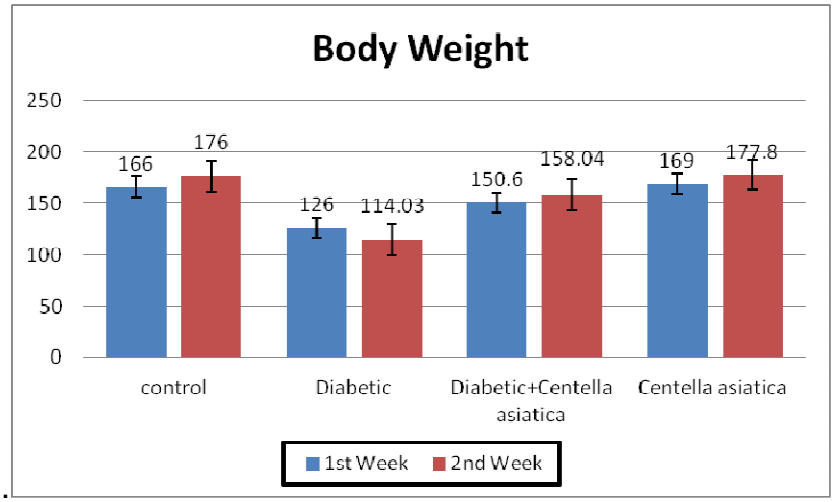

Figure 1
Effect of Centella asiatica on body weight in STZ- induced diabetic rats.

Histopathological examination of pancreatic tissues of control group and *C. asiatica* treated group(Figure 2. A , D1 & D2) showed normal pancreatic architecture; the closely packed pancreatic acini composed of pyramidal shaped cells with rounded nuclei, the pale-stained normal islets of Langerhans scattered in between acini with well preserved cytoplasm and nucleus and intact interlobular connective tissue and interlobular duct appearance. On the other hand, the pancreatic sections of the streptozotocin (STZ) diabetic rats (Figure 2. B1, 2 and 3) showed marked morphological alterations such as disturbance of the acinar pattern structure, pyknotic nuclei of some acinar cells with severe damage; congestion, dilation in interlobular pancreatic duct, blood vessels and vacuolated acne. Also, islet β-cells showed irregular outline, vacuolated cytoplasm and degenerated cells. Treatment with *C. asiatica* in diabetic rats (Group III) (Figure 2.C) showed attenuation in the previous histological alterations induced after streptozotocin treatments. Thus, the histological results support the biochemical analysis.

Figure 2

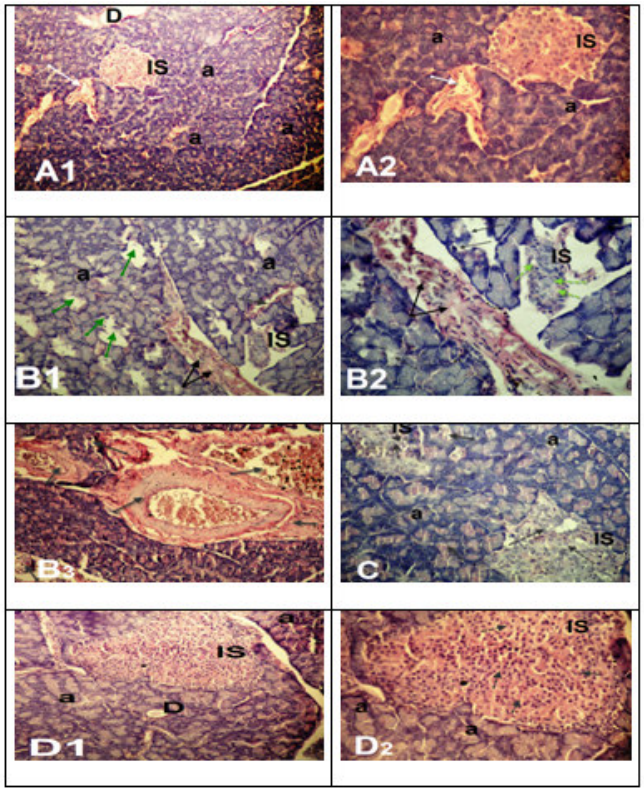

Figure 2: photomicrograph from pancreatic sections of (A, 1&2) control (group I), D1 and D2 of Centella asiatica (group IV) treated rats showing normal pancreatic architecture; the closely packed pancreatic acini composed of pyramidal shaped cells with rounded nuclei (a), the pale-stained normal islets of Langerhans (Is) scattered in between acini with well preserved cytoplasm and nucleus. B1, 2 and 3 sections in pancreatic tissue of streptozotocin (STZ) treated rats showing disturbance of the acinar pattern structure, degeneratiion of some acinar cells and vacuolated acini (green arrows); congestion, dialiation and thickening of the blood vessels and in interlobular pancreatic duct (black arrows). Islets (Is) with irregular outline, vacuolated cytoplasm (green arrows) and degeneration of β-islet cells (black dotted arrows). Histopthological alterations induced after streptozotocin treatments were markedly reduced in the groups III (C); diabetic + Centella asiatica -treated rats (H. & E. X 200 & X 400).

DISCUSSION

C. asiatica was reported to possess various types of phytochemicals such as asiaticoside, madecasoside, asiatic acids, centellin, asiaticin and cetel centellin, asiaticin and cetellicin (16, 17). All of which exert antioxidant activity that can play a role in blood glucose control regulation. In the present study, co-administration of *C. asiatica* improved hyperglycemia and hypoinsulinemia in streptozotocin diabetic rats (Table 1). These results came in accordance with several studies that found *C. asiatica* possessing significant hypoglycemic activity in glucose tolerance tests in experimental animals (18). Anderson et al. (19) revealed that the polyphenolic polymers found in herbal extract function as antioxidants, potentiate insulin action and may be beneficial in the control of glucose intolerance and diabetes. Ramachandran and Saravanan (20) stated that acetic acid (AA, a triterpenoid derivative of *C. asiatica*) improves the level of plasma insulin decreases glucose level, reverses the changes in the levels of the key carbohydrate metabolizing enzymes. In addition, the present investigation showed that, the high levels of glycosylated hemoglobin (HbAlc) in diabetic rats were significantly lowered by oral administration of *C. asiatica* (Table 1). The level of (HbA1C) has been shown to be an important parameter of chronic glycemic control in patients with DM. Decreased HbA1c levels in the treated diabetic rats could be due to an improvement in insulin secretion from the remnant pancreatic β-cells in diabetic rats, consequently, resulting in improvement in glycemic control (21). Insulin resistance is a common finding in diabetes mellitus and may serve as a measure of efficacy of therapies for diabetes mellitus and as a possible marker for risk of developing type 2 diabetes mellitus. It is characterized by a lack of the physiological response of peripheral tissues to insulin

action, leading to the metabolic and hemodynamic disturbances known as the metabolic syndrome (22). In the current study the low Quantitative Insulin Sensitivity Check Index (QUICKI) values were observed with the low observational fasting serum insulin levels (Table 1). These results are consistent with the results of Katz et al. (23) and Mack et al. (24). C-peptide and insulin are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable index of insulin secretion rather than insulin alone (25). The results of the present study demonstrated that the plasma C-peptide and insulin levels were significantly higher ($P<0.05$) in the *C. asiatica* treated group than in the untreated diabetic control group (Table 1). These results are in agreement with Mandade (26) who stated that oral administration of *C. tinctorius* crude extract to diabetic rats significantly reduced blood glucose, but increased the activities of insulin and C-peptide.Glucokinase (GK) is the first enzyme in glycolysis, glycogen synthesis, and the hexose monophosphate pathway. The reduced hepatic GK activity in diabetic rats may be due to insulin deficiency (21). In the present results oral administration of C. *asiatica* significantly increased the activity of GK compared with untreated diabetic control (Table 2). These results were consistent with those obtained by Ramachandran and Saravanan (20) who stated that, lower activity of glucokinase and hexokinase leads to impaired oxidation of glucose via glycolysis, resulting in hyperglycemia and decreased ATP production. The present measurements of α-amylase activity have shown a reduction in the enzyme activity within the first two weeks of diabetes (Table 2). While treatment with *C. asiatica* led to increase in the amylase activity. It seems that the initial drop in the amylase activity may be interpreted by the impaired pancreatic exocrine secretion due to a decrease in the insulin stimulatory action (27). The present study confirms the existence of a close functional relation between pancreatic endo- and exocrine parts, the latter being measured by serum amylase activity, in the course of alloxan-induced experimental diabetes (28). Liver glycogen level is considered as the best marker for assessing antihyperglycemic activity of any drug (29). The oral administration of *C. asiatica* showed an increased in glycogen content in the liver and muscle (Table 2). These results come in the line with the results of Gayathri et al. (30) who found that the ethanolic extract of *C. Asiatica* caused increased glycogen content in the liver compared to the glibenclamide standard. The increase in liver and muscle glycogen of diabetic rats treated with natural products may be due to the increased insulin response which in turn promotes conversion of the inactive form of glycogen to the active form and enhances conversion of blood glucose into glycogen (31). The prevention of depletion of glycogen in the liver and muscle is possibly caused by stimulation of insulin release from existing pancreatic β-cells, which enhances glycolysis (32).Furthermore, oxidative stress induced by hyperglycemia leads to the activation of stress-sensitive signaling pathways, which worsen both insulin secretion and action, and promote the development of type 2 diabetic mellitus (33). Similarly, oxidative stress and damage to the tissues and the blood in STZ-induced diabetic rats enhance glucose autoxidation, and may be a factor contributing to complications associated with diabetes. A study has reported that thiobarbituric acid reactive substances (TBARS) levels are elevated and activities of antioxidant enzymes (SOD, GPX and CAT) are significantly reduced in STZ-induced diabetic rats (34) and this is coincided with the present results, where the levels of MDA were increased and the levels of reduced glutathione (GSH) were decreased in STZ- induced diabetic rats. Oral administration of *C. asiatica* significantly increased the activities of antioxidant enzymes (SOD, GPX and CAT) which suggest that the extract may have ability to prevent the deleterious effects induced by free radicals. *C. asiatica* is well known to have a high antioxidant activity (35). The occurrence of anemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC›s membrane proteins (36). Oxidation of these proteins and hyperglycemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to hemolysis of RBC›s (37). The marked reduction in RBC, PCV and Hb was agreeing with existing literature that anemia is

a common pathophysiology associated with diabetes mellitus (38). Diabetes consist with abnormalities of hematological functions such as blood cell morphology and decreasing of red or/and white blood cell counts (39-41). Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets (42). Colak (43) also reported that diabetes mellitus causes the development of hypochromic anemia due to a fall in the iron content of the body resulting from oxidative stress associated with the condition. Following *C. asiatica* administration, the level of RBC›s was appreciably improved. This may be due to the presence of some phytochemicals that can stimulate the formation or secretion of erythropoietin. The stimulation of this hormone enhances rapid synthesis of RBC›s (44). This effect may be attributed to the ability of plant to lower lipid peroxidation level that causes hemolysis of erythrocytes (45). Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC›s and certain organs in the body. The reduction of WBC›s could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (46). The white blood counts were significantly restored to near normal values after *C. asiatica* administration. The presence of some phytochemicals in the extract with the ability to stimulate the production of white blood cells could be responsible for the observed result in the treated rats (47). The inccreases observed in RNA and DNA concentrations in diabetic rats supplemented with the gotu kola, compared to untreated diabetics, may be related to the improvement in the glycemic status of the diabetic animals and protein anabolism. The oral and topical dose administration of gotu kola in rats, increased cellular hyperplasia and collagen production were noted at the site of injury, measured by increased granulation tissue levels of DNA, protein, total collagen, and hexosamine (48).The body weight was restored in the presence of *C. asiatica* in STZ-induced diabetic rats, demonstrating its antidiabetogenic effect. The capacity of *C. asiatica* to protect against the body weight loss could be attributed to its ability to reduce hyperglycaemia. This may be achieved via the suppression of hepatic gluconeogenesis and glucose output from the liver, which is associated with the inhibition of lipolysis in adipose tissue (49). These findings were consistent with the fact that *C. asiatica* caused a reduction in the level of circulating glucagon in diabetic rats (50). It is essential to preserve glucose homeostasis, as it is a key part of the normal regulation of hepatic metabolic activities (49) and maintenance of blood glucose concentration within the normal range.Treatment with *C.asiatica* in diabetic rats group III (Figure 2.C) reversed and restored degenerative changes associated with pancreas. The result is comparable with the findings by Adewole and Ojewole (51). The findings of this reference indicate that consumption of the extract of *C. asiatica Linn.* exerts significant hypoglycemic effect in diabetic rats. Also, histopathological studies of the pancreas of diabetic treated rat show evidence of signs of regeneration of β-cells in groups receiving extracts. These findings support the traditional use of *Centella* leaves for controlling hyperglycemia in diabetics, in view of the restorative effects of the extract on pancreatic islet cells. The present findings may indicate the presence of some hypoglycemic agents in *C. asiatica Linn*, which have been concentrated in the extracts. It may have some chemical components that exert regenerative effects on β-cells, stimulate these cells to produce more insulin (pancreatotrophic action) or may have some insulin like substances. Induction of regenerative stimulus in a diabetic state triggers pancreatic regenerative processes of plants on pancreatic tissue (10). The production of lipid peroxides was significantly decreased by administration of *C. asiatica* extract. This result may be due to the active compounds of *C. asiatica Linn.* such as disulphides and their oxidized those which have been reported to have an anti oxidative effect (19). Azuma et al. (52) indicated that these compounds may contribute to the protective effects of *C. asiatica Linn.*

CONCLUSION

The *C. asiatica* has a greater restorative and protective effect on the pancreatic tissue of diabetic rats. The present findings indicate

that an aqueous extract of *C. Asiatica* is effective in the treatment of diabetes caused by STZ in rats.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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