Comparative study of *Catharanthus roseus* extract and extract loaded chitosan nanoparticles in alloxan induced diabetic rats

Md. Abul Barkat\(^1\)* and Mohd. Mujeeb\(^2\)

\(^1\)Faculty of Pharmacy, Integral University, Lucknow, U.P-226026 India.
\(^2\)Plant Tissue Culture Lab, Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062 India.

*Correspondence Info:*
Md. Abul Barkat
Faculty of Pharmacy,
Integral University, Lucknow, U.P-226026 India
E-mail: abulbarkat05@gmail.com

**Abstract**

**Objective:** The purport of this study was carried out to define the antidiabetic and \textit{in vivo} antioxidant activity of *Catharanthus roseus* var. \textit{alba} (*C.roseus var. alba*) extract loaded chitosan nanoparticles (ELCN) and methanolic leaf extract (MLE) in alloxan-induced diabetic rats.

**Methods:** The Allooxan (ALX) model for the experimental induction of diabetes in rat. Animals were allocated into seven groups of six rats each: I Normal control group (NC) rats received distilled dihydrogen monoxide 10ml/kg, II diabetic group (DC) rats received 3% v/v Tween 80 in distilled water 10ml/kg, III diabetic rats fed with glibenclamide (GLB), IV & V fed with ELCN (50 and 100 mg/kg) and VI & VII fed with MLE (200 and 400 mg/kg). One-way ANOVA followed by post hoc test was acclimated to assess the consequential difference due to administration of ELCN and MLE. For \textit{in vivo} antioxidant activity of ELCN and MLE, liver tissues were homogenized and were quantified reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were performed in NC, DC, GLB and ELCN as well as in MLE treated rats.

**Results:** In alloxan-induced diabetic rats, both the ELCN and MLE decremented blood sugar levels and body weight at the cessation of 1st, 2nd and 3rd week after test extract treatment. Antioxidant enzymes activities such as CAT, SOD and GSH levels significantly decremented in the plasma and liver of diabetic rats compared to controls. The nanonization of extract lesser the dose, increment the bioavailability and specificity of required action.

**Conclusions:** Findings of this study sanction us to establish scientifically ELCN and MLE as a potent antidiabetic agent with antioxidant effects.

**Keywords:** Alloxan, *Catharanthus roseus* var. \textit{alba}, Antidiabetic, Antioxidant enzymes and Nanonization

1. Introduction

Diabetes is a global prevalent endocrine disorder with an estimated worldwide prevalence of 246 million people in 2007 and forecasts to elevate to 300 million by 2025, and it presents a major challenge to healthcare systems around the world\(^1\). The epidemic of diabetes has been stimulating the quest for incipient concepts and targets in the treatment of this incurable disease. The worldwide diabetes has shadowed the spread of modern lifestyle and it can be linked to an incrementation over weight and sedentary population\(^2\). It is an endocrine predicated disorder of multiple etiologies characterized by hyperglycemia and hyperlipidemia. The patients suffer from diabetes experience sundry complications, such as atherosclerosis, diabetic nephropathy and neuropathy\(^3\). There are many oral hypoglycemic agents, such as sulfonylurea and biguanides, are available along with insulin for the treatment of diabetes, but these agents have paramount
side effects, and some are ineffective in chronic diabetes patients. Thus, there is an incrementing desideratum of incipient natural hypoglycemic products especially nutraceuticals with less side effects, safe, and high antihyperglycemic potential.

According to the World Health Organization (WHO) more than 80% of the people of developing countries rely on traditional medicines, mostly plant-derived drugs, for their primary health needs. The pathophysiology of diabetes involves a very intricate cascade of several interrelated mechanisms. The anterior research shows that the diabetes exhibits enhanced oxidative stress and high reactive oxygen species (superoxide, hydroxyl radical, hydrogen peroxide) in pancreatic islets due to sedulously assiduous and chronic hyperglycemia, thus depletes the activity of antioxidative bulwark system, and thus promotes free radical generation. A number of mechanisms or pathways by which hyperglycemia, the major contributing factor of incremented reactive oxygen species (ROS) engenderment, causes tissue damage or diabetic complications have been identified. Withal, reduced antioxidant levels as a result of incremented free radical engenderment in experimental diabetes have been reported. In type 1 diabetes, ROS are involved in β-cell dysfunction initiated by autoimmune reactions and inflammatory cytokines. In type 2 diabetes, ROS activate β-cell apoptotic pathways, impair insulin synthesis and withal contribute to insulin resistance. While oral hypoglycemic agents may be efficacious for glycemic control, at least in the early stages of diabetes, they do not appear to be efficacious in entirely averting the progression of ROS mediated organ damage.

Fresh leaf juice of C. roseus Linn. has been reported to reduce blood glucose in normal and alloxan diabetic rabbits. Leaves and twigs of C. roseus have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats. Diabetes mellitus is one of those chronic ailments which have been acclimated to be managed by traditional herbal medicine with over 400 plants reported to have anti-diabetic properties including Vernonia amygdalina, Grongonema latifolium, C. roseus.

The fresh juice from the flowers of C. roseus has been reported to exert antimicrobial effect. C. roseus has been shown to contain sundry constituents which are implicated for its numerous pharmacological activities.

In recent years, the nanonization of herbal medicines has magnetized much attention. Nanonization possesses many advantages, such as incrementing compound solubility, reducing medicinal doses, and amending the absorbency of herbal medicines compared with the respective crude drugs preparations. Hence, the objective of present study is to investigate the scientific substructure for the folkloric utilization of C. roseus var. alba extract and advancement in the bioavailability of C. roseus extract through nanonization technique for the treatment of diabetes.

2. Materials and Methods

2.1. Chemicals

Alloxan (Sigma–Aldrich, USA); Glucose estimation kit (Span diagnostic Ltd., Surat, India); Glibenclamide tablets. (GLB) obtained from Aventis Pharma Ltd., Mumbai, India were utilized in this study. All the other solvents and chemicals utilized for extraction were of analytical grade purchased from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India.

2.2. Collection of Plant material

The leaves of C. roseus var. alba (white variety) was taxonomically authenticated by the Prof. M. Jawaid department of botany, Faculty of Science, Jamia Hamdard, New Delhi-62. A voucher specimen (JHCP133) was deposited in college herbarium. Fresh leaves of C. roseus var. alba were collected from the Herbal Garden of University campus and the extract loaded chitosan nanoparticles (ELCN) were developed in Nanomedicine Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-62. The leaves were shade dried and grinded to coarse powder. The coarse powder was subjected to extraction with methanol by soxhlet apparatus.

2.3. Extraction

2.3.1. Preparation of leaf extract

The leaf of C. roseus var. alba was shade dried and coarsely powdered. 150g coarse powdered leaf extracted with methanol (95%) by soxhlet apparatus and extract was concentrated to dryness in vacuum, yielding a value of 10.89g (7.26% w/w) of leaf powder. The greenish brown extract of leaf was dissolved in Tween 80 of pharmacological studies.

2.3.2. Preparation of solution of extract loaded chitosne nanoparticles

An accurately weighed quantity of ELCN equivalent to approximately 1g leaf extract of C. roseus var. alba is dissolved in Tween 80 of pharmacological studies.

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2.4. Animals

Two to three month old either sex albino Wister rats of body weight 180 - 210 g were obtained from Jamia Hamdard central animal house facility (CPCSEA Regd. no. 173/ CPCSEA, dt. 18 May, 2011) acclimatized for seven days to faculty animal house, and maintained at standard conditions of temperature and relative humidity, with a 12-hour light dark cycle. Water and commercial rat feed *ad libitum* were provided. The current study was carried out with prior sanction from our Institutional Animal Ethical Committee and proposal no. 676.

2.5. Induction of diabetes to experimental animals

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) has been commonly utilized as an animal model of diabetes. The animals were fasted for 12h prior to the induction of diabetes with slight modification. Alloxan (ALX) freshly prepared in 0.5% Tween 80 was administered intraperitoneally at single dose of 140mg/kg body weight. Development of diabetes was confirmed by measuring blood glucose concentration 5 days after the administration of ALX. Rats with blood glucose level of above 200 mg/dl were considered to be diabetic and used for the studies.

2.6. Experimental design

In the present experiment, a total of 42 rats (36 diabetic surviving rats; 6 normal rats) were used. The rats were randomized into seven groups comprising of six animals in each group as given below.

- **Group I**: Normal control (NC) rats received distilled water 10ml/kg body weight, p.o.
- **Group II**: Diabetic control (DC) rats received 3% v/v Tween 80 in water 10ml/kg body weight, p.o.
- **Group III**: Diabetic rats received standard drug GLB (10 mg/kg/day, p.o.), 5 days after ALX treatment.
- **Group IV**: Diabetic rats given ELCN (50 mg/kg/day, p.o.), 5 days after ALX treatment.
- **Group V**: Diabetic rats given ELCN (100 mg/kg/day, p.o.), 5 days after ALX treatment.
- **Group VI**: Diabetic rats given MLE (200 mg/kg/day, p.o.), 5 days after ALX treatment.
- **Group VII**: Diabetic rats given MLE (400 mg/kg/day, p.o.), 5 days after ALX treatment.

2.7. Biochemical assay

2.7.1. Glucose levels

Blood samples were collected from retro-orbital plexus of each rat under mild anesthesia at 0, 1, 2 and 4hrs (Acute study) as well as on 0th, 7th, 14th and 21st days after 1 h administration (Chronic study) of ELCN and MLE. Blood glucose level was estimated by enzymatic glucose oxidase method. The reduction in blood glucose level was calculated with reverence to the initial level. The body weight of all animals was quantified on the 0, 7th, 14th and 21st days after 1h of treatment with the conveyance/GLB/extracts.

2.7.2. Glucose tolerance test

Five days before the termination of the experiment, the oral glucose tolerance test (OGTT) was performed to evaluate the ability to respond appropriately to a glucose challenge. For this purpose, overnight fasted rats (control and treated rats) were feed glucose (2g/kg body weight) orally and blood was collected at 0, 30, 60 and 120 min interval from orbital sinus for glucose estimation using a glucometer (Esprit 2, BAYER, France).

On 21st day of the study, blood samples were collected for biochemical estimations. Animals from each experimental group were starved for 16 hours and sacrificed by cervical dislocation. The liver and pancreas were removed, washed thoroughly with ice-cold saline and used for biochemical analysis.

2.7.3. Assessment of mortality rate in alloxan-induced diabetic rats

Alloxan may cause severe ketoacidosis and may lead to death of animal. In view of this the mortality rate was monitored throughout the study. The percentage of mortality was calculated at the end of each week of treatment on 7th, 14th, 21st day.

2.7.4. Antioxidant enzymes and glutathione assays in plasma and liver

2.7.4.1. Total superoxide dismutase (SOD) activity

SOD activity was assayed by the method of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM of tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, 2 μM riboflavin and 75 μM nitroblue tetrazolium (NBT). The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units per milligrams of protein.

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2.7.5.2. Catalase activity (CAT)

CAT activity was assayed by the method of Claiborne (1985)\textsuperscript{23}. Briefly, the reaction mixture consisted of 1.95 ml of phosphate buffer (0.05M, pH 7.0), 1 ml of H\textsubscript{2}O\textsubscript{2} (0.019 M) and 0.05 ml of PMS (10% w/v) in a final volume of 3 ml. Control cuvette contained all the components except substrate. Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of nanomoles H\textsubscript{2}O\textsubscript{2} consumed per minute per milligram of protein.

2.7.6.3. Glutathione levels (GSH)

GSH in tissue was assayed according to the method of Ellman (1959)\textsuperscript{24}, modified by Jollow, Mitchell, Zampaglione, and Gillette (1974)\textsuperscript{25}, based on the development of a yellow color when DTNB (5, 5-dithiobis-2 nitro benzoic acid) was added to compounds containing sulfhydryl groups; 500 μl of tissue homogenate in phosphate buffer was added to 3 ml of 4% sulfosalicylic acid. The mixture was centrifuged at 1600g for 15 min; 500 μl of supernatant was taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as milligrams per milliliter in plasma and as milligrams per milligram of protein in liver.

2.8. Histopathological examination

The pancreas, intended for histopathological evaluation by light microscopy, was abstracted and immediately preserved in 10% neutral buffered formalin, embedded in paraffin, serially sectioned at 5μm and stained with hematoxylin-eosin.

2.9 Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnett’s t-test. The changes between groups were considered significant if the P-value was less than 0.05 (P<0.05).

3. Results

3.1. Antidiabetic activity in alloxan-induced diabetic rats

Fasting blood glucose (FBG) levels were within the range of 96–99 mg/dl in normal rats. Treatment with alloxan (140 mg/kg, i.p.) had increased the blood glucose level (BGL) to a range of 250–295mg/dl after 5 days. Single dose administration of ELCN (50mg/kg) and MLE (200mg/kg) significantly (P<0.05 and P<0.01) reduced the BGL at the time intervals viz. 2 and 4 hour after treatment, in alloxan-induced diabetic rats, while ELCN (100mg/kg) and MLE (400mg/kg) as well as Glibenclamide (10mg/kg) significantly (P<0.05 and P<0.01) reduce the BGL at 1st, 2nd and 4th hour after single dose administration in alloxan-induced diabetic rats (Table 1).

| Treatment     | Dose (mg/kg) | 0h     | 1h     | 2h     | 4h     |
|---------------|--------------|--------|--------|--------|--------|
| Normal Control| -            | 97.11 ± 0.49** | 97.15 ± 0.67** | 96.85 ± 0.61** | 98.17 ± 0.71** |
| DC (3%v/v Tween 80) | 10ml/kg | 262.16 ± 1.55 | 267.55 ± 1.75 | 270.09 ± 2.25 | 268.28 ± 3.06 |
| GLB 10mg/5mg  | 261.67 ± 1.56 | 255.16 ± 3.82** | 210.06 ± 0.85** | 181.53 ± 1.34** |
| ELCN 50mg/kg  | 260.12±3.38  | 264.34± 3.01  | 260.72±4.31*  | 238.03±0.45** |
| ELCN 100mg/kg | 263.85±1.18  | 258.27± 2.65* | 260.72±4.31*  | 237.44±0.88** |
| MLE 200mg/kg  | 200mg/kg     | 259.88±1.16  | 259.29±2.35  | 261.13±5.29  | 255.13±2.28* |
| MLE 400mg/kg  | 400mg/kg     | 261.12±0.84  | 258.97±1.56* | 259.86±2.27* | 248.46±0.75** |

The data are expressed in mean ± S.E.M. n = 6 in each group. P values were analysed using One-way ANOVA followed by post hoc Dunnett’s test.

*p < 0.05 compared with corresponding value of diabetic control animals and

**p < 0.01 compared with corresponding value of diabetic control animals.

Repeated dose administration with ELCN (50mg and 100) and MLE (200 and 400 mg/kg) had progressively reduced the BGL in a dose dependent manner over a period of 3 weeks (Table 2). However, animals treated with both the doses of ELCN showed a significant decrease (P<0.05 and P<0.01) in BGL on 4th, 7th, 14th and 21st days of treatment.
when compared to other groups of animals; similarly animals treated with both the doses of MLE also showed a significant ($P<0.05$ and $P<0.01$) decrease in BGL compare to other groups.

The data in Table 2 show that the ELCN treatment for 21 days in diabetic rats has caused a reduction in BGL when compared to MLE, indicating the potency of ELCN at lower dose. These results indicate that the ELCN and MLE both possess antidiabetic activity on repeated administration in alloxan induced diabetic rats. The nanonization of leaf extract causes the dose reduction of extract, and becomes more targeted towards their active site.

### Table 2. Effect of ELCN and MLE on blood glucose level in ALX-induced diabetic rats (Chronic Study).

| Treatment       | Dose (mg/kg) | Blood glucose level (mg/dl) |
|-----------------|--------------|----------------------------|
|                 |              | 0 day          | 7th days          | 14th days          | 21st days         |
| Normal Control  | -            | 96.58 ± 0.31** | 97.57 ± 0.59**   | 98.06 ± 0.54**    | 97.73 ± 0.63**   |
| DC (3%v/v Tween 80) | 10ml/kg     | 252.83 ± 4.81  | 263.13 ± 5.65    | 280.51 ± 3.62     | 292.25 ± 1.62    |
| GLB             | 10mg/kg      | 263.16 ± 2.06  | 119.93 ± 3.80**  | 105.29 ± 2.84**   | 95.36 ± 1.52**   |
| ELCN            | 50mg/kg      | 256.24±2.81    | 249.91±3.14*     | 235.74±3.04**     | 200.37±4.05**    |
| ELCN            | 100mg/kg     | 262.02±3.87    | 249.91±3.14*     | 167.89±1.83**     | 148.92±1.87**    |
| MLE             | 200mg/kg     | 260.49±2.64    | 119.93±3.80**    | 105.29±2.84**     | 95.36±1.52**     |
| MLE             | 400mg/kg     | 259.72±1.99    | 201.34±2.44**    | 184.41±3.79**     | 171.75±4.55**    |

The data are expressed in mean ± S.E.M. n = 6 in each group. P values were analysed using One-way ANOVA followed by post hoc Dunnett’s test.

* $p < 0.05$ compared with corresponding value of diabetic control animals.

** $p < 0.01$ compared with corresponding value of diabetic control animals.

### 3.2. Effect of ELCN and MLE on Body Weight in Experimental Groups

Treatment with alloxan (140 mg/kg, i.p.) had significantly decreased the body weight at the end of 7th, 14th and 21st days as compared to normal animals. Repeated administration of Glibenclamide (10mg/kg) had prevented the reduction in body weight on 14th and 21st day in diabetic rats. These results concluded that ELCN (100mg/kg) were able to significantly ($P<0.05$ and $P<0.01$) inhibit the body weight only after the 14th day and MLE 400 mg/kg on 7th day, whereas with ELCN (50mg/kg) and MLE (200 mg/kg), a significant ($P<0.01$) decrease in body weight was seen on the 21st day (Table 3).

### Table 3. Effect of ELCN and MLE in the body weight of alloxan-induced diabetic rats.

| Treatment       | Dose (mg/kg) | Body weight (g)  |
|-----------------|--------------|------------------|
|                 |              | 0 day          | 7th days          | 14th days          | 21st days         |
| Normal Control  | -            | 181.77 ± 0.94** | 184.58 ± 1.76**  | 193.71 ± 1.52**   | 196.55 ± 2.27**  |
| DC (3%v/v Tween 80) | 10ml/kg     | 199.17 ± 1.63  | 173.98 ± 1.54    | 171.24 ± 2.23     | 161.52 ± 3.01    |
| GLB             | 10mg         | 192.03 ± 1.39  | 178.92 ± 3.91    | 185.48 ± 2.49**   | 189.11 ± 3.04**  |
| ELCN            | 50mg/kg      | 193.98±1.81    | 176.65±1.53      | 179.24±1.79       | 181.97±2.17**    |
| ELCN            | 100mg/kg     | 190.10±1.86    | 177.97 ± 2.09    | 182.14±1.03*      | 187.07±2.76**    |
| MLE             | 200mg/kg     | 192.61±3.13    | 178.94±3.28      | 179.05±2.81       | 182.03±1.38**    |
| MLE             | 400mg/kg     | 191.33±2.56    | 184.27±2.24*     | 182.41±2.08*      | 185.83±3.19**    |

The data are expressed in mean ± S.E.M. n = 6 in each group. P values were analysed using One-way ANOVA followed by post hoc Dunnett’s test.

* $p < 0.05$ compared with corresponding value of diabetic control animals.

** $p < 0.01$ compared with corresponding value of diabetic control animals.

Single administration of alloxan (140 mg/kg, i.p.) had produced mortality of 38% over a period of 3 weeks. Repeated administration of Glibenclamide (10 mg/kg) had prevented the mortality in alloxan-induced diabetic rats throughout the study. Repeated dose administration of ELCN (50 and 100 mg) showed mortality of 11% while MCE at 400 mg/kg dose level showed 27% mortality rate at the end of 21st days. These results indicated that glibenclamide and C.rosea var. alba extract loaded chitosae nanoparticles and methanolic leaf extract could protect the animals against alloxan induced mortality.
3.3. The effect of ELCN and MLE on Antioxidant enzyme activities and glutathione levels in plasma and liver

The antioxidant enzyme activities (CAT and SOD) and GSH levels in the plasma and liver of NC and tested groups are shown in Table 4. In DC group, a significant \((P<0.05\) and \(P<0.01\)) decrease of GSH levels and CAT and SOD activities was observed in plasma \((-42\%, -74\%, -40\%)\) and liver \((-41\%, -32\%, -49\%)\), respectively, as compared to the NC group. In ELCN (50 and 100mg/kg), MLE (200 and 400mg/kg) and GLB treated group, GSH levels, CAT, and SOD activities in plasma (44%, 2%, 44% and 94%, 27%, 132%), (20%, −1%, 20% and 78%,18%,104%) and (114%, 26%, 141%) and liver (39%, 127%, 29% and 81%, 179%, 69%), (25%, 91%, 18% and 63%, 144%, 47%) and (111%, 204%, 79%) respectively, as compared to those of the DC group.

Table 4. Effect of ELCN and MLE on CAT, SOD and GSH levels in ALX-induced diabetic rats.

| Parameters and Treatment | NC       | DC       | GLB      | LCN(50mg) | ELCN(100mg) | MLE(200mg) | MLE(400mg) |
|--------------------------|----------|----------|----------|-----------|-------------|------------|------------|
| SOD                      |          |          |          |           |             |            |            |
| Plasma                   | 16.14 ± 0.07** | 6.49 ± 0.19** | 15.67 ± 0.19** | 12.22 ± 0.11** | 15.05 ± 0.12** | 10.06 ± 0.15** | 13.22 ± 0.37** |
| Liver                    | 16.98 ± 0.13** | 8.39 ± 0.25** | 15.03 ± 0.19** | 10.83 ± 0.39** | 14.17 ± 0.19** | 9.87 ± 0.26** | 12.35 ± 0.22** |
| CAT                      |          |          |          |           |             |            |            |
| Plasma                   | 6.56 ± 0.27** | 4.88 ± 0.22 | 6.15 ± 0.21** | 4.97 ± 0.23  | 6.20 ± 0.23** | 4.84 ± 0.18 | 5.74 ± 0.18* |
| Liver                    | 5.21 ± 0.21** | 1.69 ± 0.18 | 5.13 ± 0.19** | 3.83 ± 0.32** | 4.72 ± 0.26** | 3.23 ± 0.13** | 4.13 ± 0.24** |
| GSH                      |          |          |          |           |             |            |            |
| Plasma                   | 8.94 ± 0.13** | 3.72 ± 0.27 | 7.97 ± 0.08** | 5.35 ± 0.17** | 7.21 ± 0.11** | 4.47 ± 0.21 | 6.62 ± 0.21** |
| Liver                    | 7.44 ± 0.19** | 3.08 ± 0.15 | 6.49 ± 0.21** | 4.28 ± 0.14** | 5.57 ± 0.21** | 3.86 ± 0.12 | 5.03 ± 0.076** |

The data are expressed in mean ± S.E.M. \(n = 6\) in each group. P values were analysed using One-way ANOVA followed by post hoc Dunnett’s test.

*\(p < 0.05\) compared with corresponding value of diabetic control animals.

**\(p < 0.01\) compared with corresponding value of diabetic control animals.

3.4. Light microscopy study of pancreas tissue

The histological examination of the NC and ELCN (100mg/kg) and MLE (400mg/kg) treated groups showed normal β-cell architecture. Alloxan administration elicited significant morphological changes in DC rats with severe injury of pancreatic β-cells, such as decreasing the islets cell numbers, cell damage, and cell death (Fig. 1A–D).

Fig 1. Pancreas histological sections (hematoxylin and eosin, × 400). (A) NC group. (B) DC group. (C) ELCN (100 mg/kg) group. (D) MLE (400 mg/kg) group.
4. Discussion

The diabetes inducing agent alloxan is a hydrophilic and chemically unstable derivative of pyrimidine, which is toxic to pancreatic β-cells because it can engender toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine. Alloxan causes a massive reduction in insulin release by the ravagement of the β-cells of the islets of langerhans, inducing hyperglycaemia. Elevated blood glucose levels cause an incrementation in oxygen free radicals in diabetes, which engenders free radicals due to auto oxidation. In the present work, participation of free radicals in progression of disease and protective effects of ELCN and MLE has been examined. The administration of ELCN and MLE for 21 days showed paramount antidiabetic and antioxidant activities in ALX induced diabetic rats. Traditional herbal medicines are utilized throughout the world for a range of diabetic presentations the study of such plant derived medicines might offer a natural key to unlock a diabetologist’s pharmacy for the future.

In the present study, we investigated whether the *C. roseus var. alba* extract loaded chitosan based nanoparticle at lesser dose shows antidiabetic and antioxidant action in alloxan-diabetic rats as compared to the higher dose of methanolic extract. The most important result of the present study was that rats, fed ELCN (50 and 100mg/kg) were able to partly recover from alloxan-induced diabetes within a short time and at lesser dose compared with rats fed MLE (200 and 400mg/kg). Interestingly, such an effect could be related to the partial regeneration or preservation of pancreatic β-cell mass after alloxan treatment. Indeed, at the end of the experiment, pancreatic β-cell mass in the ELCN (100mg/kg) group was similar to that of the NC group. In parallel, plasma insulin level significantly decremented in the DC group compared with the ELCN and MLE animals. That denotes the transmutations in insulin may establish transmutations in hepatic glycogen content and lead to the regulatory effect of ELCN and MLE on glucose metabolism in alloxan-induced diabetic rats and substantiated a defect in pancreatic β-cell function and/or a decremented β-cell mass, as shown in the histological examination of different diabetes pancreatic sections. The major antioxidant enzymes, including SOD, CAT, and GSH, are regarded as the first line of the antioxidant bulwark system against ROS engendered in vivo during oxidative stress and act cooperatively at different sites in the metabolic pathway of free radicals.

A marked depletion in the GSH, SOD and CAT content of liver was observed in diabetic control rats (Table 4). Furthermore, ELCN and MLE treatment showed a significant restoration in GSH, SOD and CAT content of diabetic rats. GSH fend the cellular system against the toxic effects of lipid peroxidation. The present data denote that ALX induced diabetes disrupts actions of antioxidant enzymes. The decremented activities of these enzymes may be due to the engenderment of ROS such of superoxide (O$_2^-•$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH) that reduces the activity of these enzymes. The body shows its own bulwark mechanism to obviate and neutralize the free radical-induced damage. The endogenous antioxidant enzymes such as SOD and CAT constitute a mutually auxiliary team of bulwark against ROS.

In the case of diabetes, the balance between ROS engenderment and these antioxidant defenses may be disoriented, resulting in oxidative stress which, through a series of events, perturbs the cellular functions leading to hepatic necrosis, for example. The decremented activity of SOD and CAT point out the tissues damage in the diabetic rats. ELCN and MLE group showed a paramount amelioration in the level of these enzymes as compared to DC group, which betokens the antioxidant activity of the extract loaded chitosan based nanoparticle and methanolic leaf extract. Apart from SOD and CAT the non enzymic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of GSH has been shown to be associated with an enhanced toxicity to chemicals, including diabetic status. In the present study, depletion of GSH level in plasma and hepatic tissue was observed in DC group. The amendment in plasma and liver GSH level in the ELCN rats may be due to the novo GSH synthesis or GSH regeneration. The GSH plays a essential protective role as a scavenger of free radicals that coalesce with non-protein thiols at the GSH reactive center to abolish free radical toxicity. ELCN, MLE and glibenclamide treatment significantly raised the antioxidant level (GSH) and antioxidant enzyme activity (SOD, CAT) in a dose-dependent manner. Likewise, ELCN and MLE exhibited the same antioxidation effects as glibenclamide at the dose of 10mg/kg b.w.

In the present study, ELCN and MLE potentiated the enzymatic and non-enzymatic antioxidant activities. Herbal drugs of natural inception were pellucidly denoted as a promising avenue for the aversion of chronic diseases. The finding of the present study shows a number of positive effects of ELCN and MLE on rats with ALX-induced perturbances in glucose tolerance and antioxidant status. Thus, ELCN and MLE is salutary in the control of diabetes and oxidative stress by activation of enzymatic and non enzymatic antioxidants. In conclusion, the result of the present study denotes that ELCN and MLE may have active principle(s) that exerts antidiabetic and antioxidant activities. However, more efforts are still
needed for the isolation, characterization and biological evaluation of the active principle(s) of the *C. roseus* var. *alba* extract.

In conclusion, our data suggest that treatment with the ELCN and MLE partly preserved pancreatic function and ameliorated peripheral glucose in alloxan induced diabetic rats. The identification of the active components in such extract, traditionally utilized in folk medicine to treat arterial hypertension and/or diabetes in Mediterranean countries, may well contribute to our cognizance of their precise molecular effects.

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