



Original Research Article (Experimental)

Cocos nucifera and metformin combination for modulation of diabetic symptoms in streptozotocin induced diabetic rats

Ginpreet Kaur^{a,*}, Himanshu Sankrityayan^a, Deepashree Dixit^a, Priyanka Jadhav^b^a Department of Pharmacology, Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM'S NMIMS, Mumbai, 400056, India^b CRC Pharma, 333 Littleton Road, Parsippany, NJ, 07054, USA

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ABSTRACT

Background: *Cocos nucifera*, belonging to Arecaceae family, holds quite an importance in the Indian traditional medicinal system. *C. nucifera* inflorescence (Cnl) has been reported in the literature to be useful in the treatment of diarrhoea, dysentery, diabetes, and dyspepsia. In this study, we aimed to evaluate the efficacy of Cnl as an adjuvant with metformin in ameliorating Type-2 diabetes mellitus (T2-DM).

Objectives: To evaluate antidiabetic activity of Cnl in combination with metformin in Streptozotocin (STZ) induced diabetic rats.

Materials and methods: Diabetes was induced in male Wistar rats using streptozotocin (45 mg/kg; i.p.). Plasma glucose level (PGL) was estimated after 72 h of STZ injection. Ethanolic extract of Cnl (250 mg/kg and 500 mg/kg) *per se* and in combination with metformin (22.5 mg/kg) was administered orally once daily to rats for a period of 28 days. PGL level was estimated on 7th, 14th and 21st day followed by Oral Glucose Tolerance Test (OGTT) and PGL both on the 28th day of treatment. DPPH assay was performed to evaluate antioxidant activity of Cnl extract.

Results: Extract of Cnl (250 mg/kg and 500 mg/kg alone and the combination of extract (250 mg/kg) along with metformin (22.5 mg/kg) significantly decreased PGL ($p < 0.0001$) on 7th, 14th, 21st and 28th days. Histopathological analysis of pancreatic tissue showed that treatment with Cnl extract *per se* and in combination with metformin improved the damaged architecture of pancreas.

Conclusion: The combination therapy of Cnl and metformin produced a significant antidiabetic effect than that of the extract alone and provides a scientific rationale for their use in antidiabetic therapy as an adjuvant.

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1. Introduction

Diabetes mellitus (DM) is a chronic condition characterized by an implied disturbed glucose metabolism due to either insulin deficiency or impaired insulin functioning or both [1]. It leads to several complications such as diabetic nephropathy, atherosclerosis, neuropathy and retinopathy [2]. In the predictable future, DM is probably going to be the leading cause of morbidity and mortality globally.

Currently oral hypoglycemics and insulin are employed for the treatment of diabetes, but none of them are without side effects viz. prolonged use of insulin may lead to insulin resistance, atrophy of

brain, anorexia etc [3]. Intricate aetiology of diabetes has led to shifting pattern of treatment from monotherapy to combination therapy [4]. A number of studies corroborate to the fact that combination therapy of synthetic drug with herbal drug is superior to the single therapy approach in many cases [3]. As many as 400 plants have been assessed for their therapeutic use in diabetes [4]. Metformin, one of the most commonly used oral hypoglycemic was synthesized based on an herbal prototype molecule of French Lilac (*Galega officinalis*) [5].

Cocos nucifera belonging to the Arecaceae family holds quite an importance in the Indian traditional medicinal system. *C. nucifera* inflorescence (Cnl) has been reported in literature to be useful in treatment of diarrhoea, dysentery, diabetes, and dyspepsia [6].

C. nucifera which is commonly used in diet is one of the promising herbs for the treatment of diabetes mellitus. According to a

* Corresponding author.

E-mail: ginpreet.aneja@gmail.com

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previous study, the fruit of *C. nucifera* has been found therapeutically effective in cardio-metabolic disorders [7]. It grows up to 30 m tall and has a smooth trunk [8]. It is mostly found in the tropical and subtropical areas and has several health beneficial effects such as antidotal, antiseptic, antitumour, bactericidal, depurative, antihelminthic, aphrodisiac, astringent, diuretic, refrigerant, stomachic, styptic, suppurative, vermifuge, antioxidant, vasorelaxant and antihypertensive [9]. The peculiar floral arrangement in the coconut palm is called the inflorescence. The inflorescence of this coconut palm is a spadix which develops in the axil of the leaf. Inflorescence of coconut is composed of amino acids, tannins, sugars and phenolic compounds [10]. Amino acids have the potential of rejuvenation of β cells of islets of Langerhans [11–13]. However, polyphenols and polyphenol-rich products modulate lipid and carbohydrate metabolism, mitigate hyperglycemia, dyslipidemia and insulin resistance, improve metabolism of adipose tissue and oxidative stress and stress sensitive signalling pathways and inflammatory process [14–16].

Hence, the present study was envisaged to evaluate the pharmacodynamic interaction of the extract of Cnl in combination with metformin in streptozotocin (STZ) induced diabetic rats. In addition to this, lipid as well as kidney profile were evaluated. The effect of exogenous glucose uptake was estimated through oral glucose tolerance test (OGTT). Further, histopathology was performed to check the effect of treatment on pancreas.

2. Materials and methods

2.1. Chemicals

STZ was purchased from SRL Laboratories, India. Metformin was received as a gift sample from USV Ltd, Mumbai, India. Glucose estimation kit, Triglyceride Estimation Kit, Total Cholesterol Estimation Kit, Low Density lipoprotein (LDL) direct kit and High density lipoprotein (HDL) direct kit were procured from Transasia Bio-medicals Ltd, Mumbai, India. All the chemicals used were of analytical grade.

2.2. Animals

Forty two male Wistar rats were purchased from Bombay Veterinary College and were acclimatized for a week in the animal house of SPP School of Pharmacy & Technology Management SVKM'S NMIMS. Meanwhile, the animals were kept in standard conditions of temperature (25 ± 2 °C) and relative humidity ($55 \pm 10\%$). They were exposed to a 12 h dark and 12 h light cycle and had free access to standard rat chow and drinking water. The protocol for the study was approved by the Institutional Animal Ethics Committee of SVKM'S NMIMS (CPCSEA/IAEC/SPTM/P-04/2014).

2.3. Plant material

Unopened young inflorescence of coconut was harvested from Vile Parle (East) Mumbai. The fresh specimen of inflorescence was authenticated from St. Xavier's college, Mumbai. The inflorescence was dried well at 45 °C followed by pulverization by electric laboratory grinder.

2.4. Preparation of Cnl extract

Cnl was prepared using ethanol 95% v/v. The extraction was carried out in an ice bath. The extract was prepared by soaking 50 g

of oven dried powdered Cnl in ethanol and was stirred occasionally. The suspension was filtered and the residues were re-extracted. The extract was concentrated using a rotary evaporator with temperature maintained such that it did not exceed 45 °C. The yield of dried material was found to be 15% w/w. The extract was stored at 2–8 °C until used [7].

2.5. In vivo studies

The acute single dose oral toxicity was studied in Swiss albino mice as per OECD guideline 423. The extract was administered orally at dose of 2000 mg/kg. Vehicle (0.5% w/v) was administered to control group. The general behaviour of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Changes in the normal activity of mice and their body weights, food and water intake were monitored and the time at which signs of toxicity or death appeared recorded.

2.5.1. Induction of diabetes

Diabetes was induced in male Wistar rats by a single intraperitoneal injection of freshly prepared solution of STZ (45 mg/kg) after overnight fasting. STZ was dissolved in citrate buffer (pH = 4.5) [17].

The animals were divided into seven groups and were dosed as per the following scheme daily for a period of 28 days; all the treatments were administered intra-gastric using a feeding needle.

Group I = Normal Control.

Group II = STZ (45 mg/kg) treated rats.

Group III = Metformin (22.5 mg/kg) treated STZ induced diabetic rats.

Group IV = Cnl (250 mg/kg) treated STZ induced diabetic rats.

Group V = Cnl (500 mg/kg) treated STZ induced diabetic rats.

Group VI = Cnl (250 mg/kg) + Metformin (22.5 mg/kg) treated STZ induced diabetic rats.

Group VII = Cnl (500 mg/kg) + Metformin (22.5 mg/kg) treated STZ induced diabetic rats.

2.5.2. Biochemical analysis

The animals were bled from retro-orbital sinus at specific time intervals of 0, 14 and 28 days post diabetes induction. Plasma was separated from the collected blood samples and subjected to determination of plasma glucose levels (PGL). On day 28, lipid profile, and kidney profile were estimated using the bioanalytical kits.

2.5.3. Oral glucose tolerance test (OGTT)

On completion of 28 days of dosing, all rats were fasted for 14 h before being subjected to OGTT by administering glucose solution 2 g/kg by oral route. Blood was withdrawn from retro-orbital sinus at 15, 30, 60 and 120 min after glucose load from all animals to determine glucose tolerance. The plasma glucose level was estimated using bioanalysis kits.

2.5.4. DPPH assay

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) assay is based on radical scavenging activity of the antioxidant. It involves transfer of an electron which leads to formation of violet coloured solution in ethanol. When a sample with antioxidant property is mixed with it, it gets reduced forming a colourless ethanol solution. 10 mg of

DPPH was weighed and dissolved in methanol. Ascorbic acid was used as a standard. DPPH was mixed with different concentrations of Cnl extract and ascorbic acid standard solutions. These solutions were kept in the dark for 30 min and absorbance was measured at 517 nm. Percentage inhibition of both sample and standard were obtained and IC50 was calculated.

2.5.5. Histopathological analysis

The animals were sacrificed at the end of the experimental period followed by fixation of liver, kidney and pancreas in 10% neutral buffered formalin for routine histopathological examination. The block of tissues were prepared by embedding in paraffin, sectioned and finally stained with haematoxylin and eosin (H&E) and were examined under light microscope. Evaluation of histopathology was performed at Unique biodiagnostic, Mumbai.

2.6. Statistical analysis

Results were expressed as mean \pm SEM. Statistical significance of reduction in plasma glucose level and other biochemical parameters were determined by One way ANOVA followed by Bonferroni test, values with $p < 0.05$, $p < 0.0001$ and $p < 0.001$ were considered as statistically significant. GraphPad Prism version 5.0, GraphPad Software Inc., was used for statistical analysis.

3. Results

3.1. Toxicity study

No signs of toxicity were observed at the dose of 2000 mg/kg Cnl extract. Similarly no significant change in body weight or consumption of feed and water was evident during the 14 days observation.

3.2. Effect of treatment on PGL and lipid profile

Figs. 1 and 2 depict that the plasma glucose, triglyceride, cholesterol, LDL levels in group treated with STZ were higher as

compared to normal control. Cnl extract significantly reduced PGL both *per se* and in combination with metformin after 28 days of treatment period. We also noticed that the combination group was more effective in lowering triglycerides, LDL, and PTC (plasma total cholesterol) levels when compared to extract or metformin alone.

3.3. Effect of treatment on creatinine level

The effect of the treatment on kidney was evaluated as shown in Fig. 3. Oxidative stress generated in diabetes due to reactive oxygen species results into damage of renal system. Elevated levels of creatinine are considered as a marker of impaired kidney function. The potential of polyphenols and flavonoids to reduce the oxidative stress, results into restoring kidney dysfunction which was observed to be the greatest in group VII.

3.4. Oral glucose tolerance test (OGTT)

In Fig. 4, the levels of blood glucose at specific time intervals after the oral administration of glucose (2 g/kg body weight) in normal and experimental groups of rats are depicted. In control rats, the blood glucose level reached the maximum peak at 20 min after an oral glucose load and then it gradually reverted to near normal levels after 60 min. In STZ-induced diabetic rats, peak increase in blood glucose concentration was observed at 20 min and remained at a plateau over the next 60 min. Oral administration of the combination of extract and metformin on STZ-induced diabetic rats showed significant decrease in blood glucose concentration at 20 and 60 min suggesting an improvement in glucose homeostasis.

3.5. DPPH assay of ethanolic extract of *C. nucifera* inflorescence

It was found that Cnl has antioxidant properties since there is an increase in percentage inhibition of DPPH free radicals with an increase in the concentration of the extract. IC50 for the extract was calculated to be 109 μ g/ml (Fig. 5).

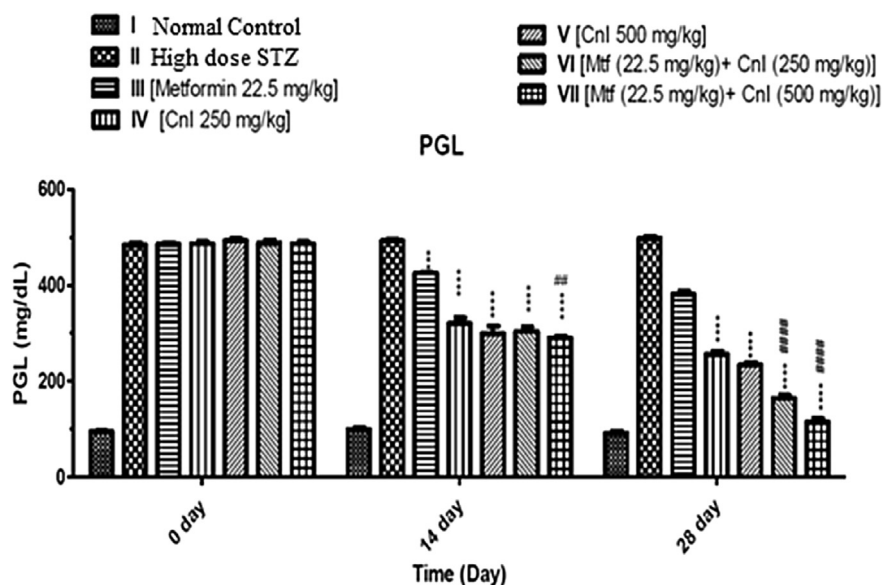


Fig. 1. Effect of Cnl extract both *per se* and in combination with metformin on plasma glucose level. Values are expressed as mean \pm SEM, **** $p < 0.001$, *** $p < 0.001$ were compared with the high dose STZ (positive) control group, ## $p < 0.05$ and #### $p < 0.0001$ were compared with metformin group.

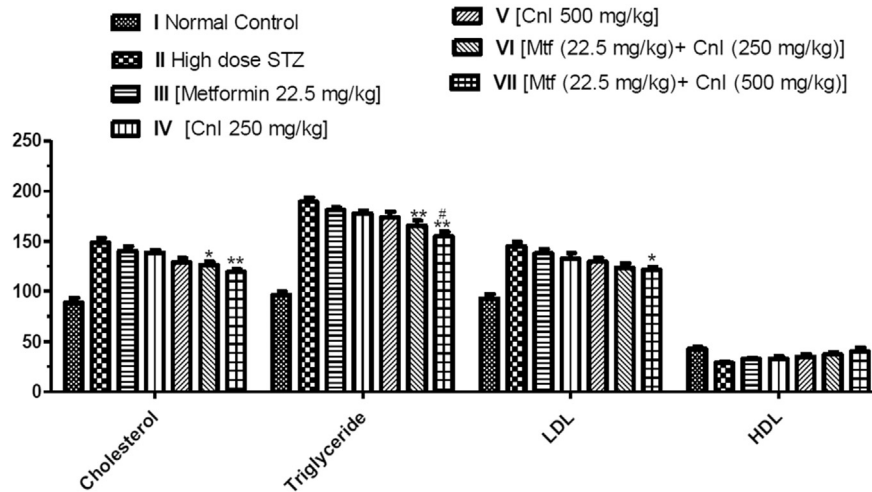


Fig. 2. Effect of Cnl extract both *per se* and in combination with metformin on lipid profile. Values are expressed as mean \pm SEM, * p < 0.05, ** p < 0.01 were compared with the high dose STZ (positive) control group, # p < 0.05 were compared with metformin group.

3.6. Histopathological analysis

In the histopathology study (Fig. 6), degenerative changes, hyalinization, necrosis, fibrosis, atrophy comparative presence of islets of Langerhans along with the size were observed. The comparative presence of islets of Langerhans and their size was also found to be reduced in diabetic control. However, by treatment of Cnl extract alone and in combination with metformin exhibited the curative effect on STZ induced pancreatic cytotoxicity. Histopathological analysis of pancreatic tissue showed that treatment with Cnl extract alone and in combination with metformin diabetic rats ameliorated the altered architecture and number of islets, restored pancreatic tissue integrity and was able to regenerate the STZ damaged pancreatic β cells.

4. Discussion

Diabetes is deliberated to be one of the utmost common chronic diseases globally. There is a rising scientific and public concern in linking oxidative stress with DM. Hyperglycaemia is responsible for induction of free radicals; it damages the endogenous antioxidant resistance system in patients [18].

For evaluation of antidiabetic potential of any herbal or synthetic compound, STZ induced diabetes rat model is the most commonly preferred method. STZ inhibits DNA synthesis in mammalian and bacterial cells. It prevents cellular reproduction with a considerably lesser dose than the dose necessary for

preventing the substrate connection to the DNA or obstructing many of the enzymes involved in DNA synthesis. Though STZ stops entrance of cells into mitosis but no singular phase of the cellular cycle is specifically sensitive to its destructive effects. STZ induces pancreatic swelling and causes degeneration in islets of Langerhans beta cells and induces experimental diabetes. It also modifies normal metabolism in diabetic rats in comparison with normal rats. Consumption of water and food, serum glucose and volume of urine increase in diabetic animals in comparison with normal rats but the levels of serum insulin, C-peptide and body weight decrease [19].

Herbs are an important dietary source of necessary nutrition and can be explored for potentially bioactive constituents for the development of novel therapeutic agents [11]. In fact as much as 25% of the modern synthetic drugs are derived from herbal sources [20].

The phytochemical screening of young inflorescence of coconut revealed the presence of phenolic acids, flavonoids and resins at high concentrations. Macronutrient investigation exhibited presence of carbohydrates at a high concentration and proteins at a moderate concentration. Phytochemicals have received a pronounced amount of attention because of their antioxidant effect. DPPH scavenging activity of Cnl methanol fraction has been observed to have a high antioxidant potential. Qualitative analysis of the Cnl methanol and ethanol extracts has revealed the presence of proteins, carbohydrates, phenolic compounds, flavonoids, alkaloids, tannins and resins [6]. Considering the rich

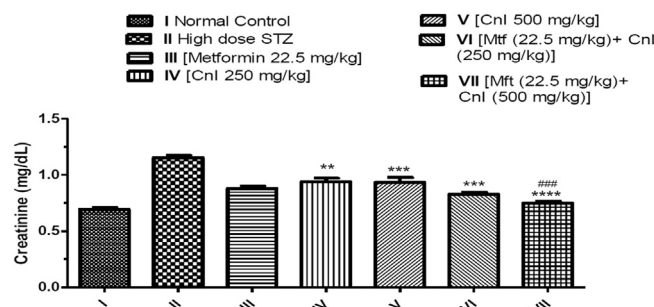


Fig. 3. Effect of Cnl extract both *per se* and in combination with metformin. Values are expressed as mean \pm SEM, ** p < 0.01, *** p < 0.001 were compared with the high dose STZ (positive) control group, ### p < 0.001 were compared with metformin group.

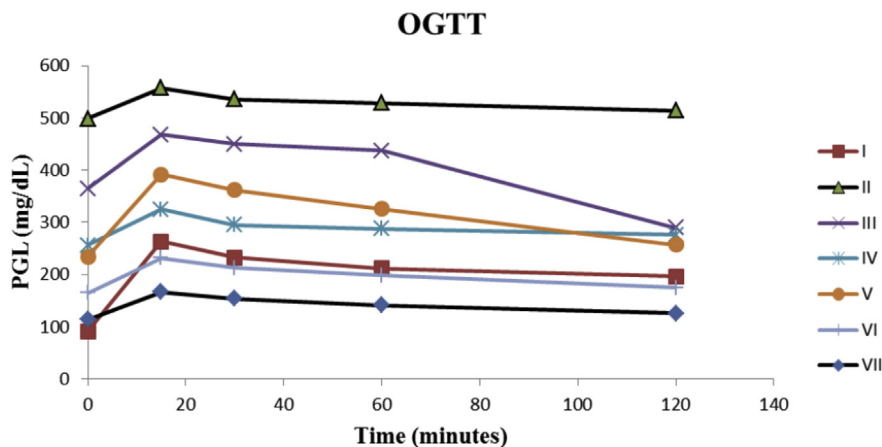


Fig. 4. Effect of Cnl extract both *per se* and in combination with metformin on OGTT on day 28.

content of flavonoids in Cnl, the hypoglycaemic activity was attributed to the antioxidant activity of bioflavonoids. The possible mechanism of action of Cnl in diabetes mellitus may be due to increased glycolysis at peripheral tissues by potentiating the action of insulin at the target cell similar to biguanides [12]. Another research indicates that owing to rich arginine contents, *C. nucifera* may be improving the pancreatic functions by regenerating cells and thus restoring the carbohydrate metabolism to the normal levels [21].

Coconut water exhibited hypoglycaemic and antioxidant potential in experimental diabetes [22]. In another study, coconut water was able to reduce plasma HbA1C and urea levels in an alloxan induced rat model of diabetes mellitus. It also helped in maintaining blood sugar levels and increasing body weight [23].

The resourceful coconut tree aptly called “*Kalpataru*” is a source of a number of useful compounds. Medical research has proven it to be beneficial in various diseases. The health benefits of *C. nucifera* are undeniably worth an extensive investigation [24].

In the present study, STZ induced diabetic rats when treated with the combination of Cnl extract and metformin resulted into decrease in the elevated plasma glucose level, triglyceride cholesterol and LDL level. However, increase in the HDL level was observed as compared to high dose STZ diabetic rats. The reduction in plasma glucose level in group VII (metformin 22.5 mg/kg, extract 500 mg/kg) was greater than individual groups. Therefore,

it can be concluded that additive effect is observed in the combination group. The possible mechanism for the therapeutic activity is, metformin increases the peripheral uptake of glucose in the presence of insulin. The extract has curative action on pancreatic cytotoxicity and hyperglycaemia by repairing and rejuvenating β cells, inhibiting α glucosidase and reducing elevated oxidative stress.

We also evaluated renal parameters since diabetes is also associated to nephropathy. There is a study which shows that coconut water is effective in ameliorating diabetic nephropathy [25].

Estimation of creatinine level is one of the indicators of kidney disorder. We found that Cnl was effective in ameliorating the increased creatinine levels. As the extract has shown to have the antioxidant activity, oxidative stress induced in the diabetes got reduced. In addition, the results of OGTT revealed that high dose STZ rats showed significant impairment in glucose tolerance to exogenously administered glucose (2 g/kg) as evident from elevated glycaemic levels at 30, 60, 90, and 120 min after glucose challenge, compared with normal control. Treatment with Cnl and metformin (22.5 mg/kg and 500 mg/kg) once daily for 4 weeks significantly decreased plasma glucose levels of high dose STZ diabetic rats. Furthermore, combination of Cnl and metformin significantly improved glucose tolerance to exogenously administered glucose (2 g/kg) after 60, 90, and 120 min interval on OGTT in high dose STZ induced diabetic rats compared with the untreated normal control group. Lastly, the histopathological

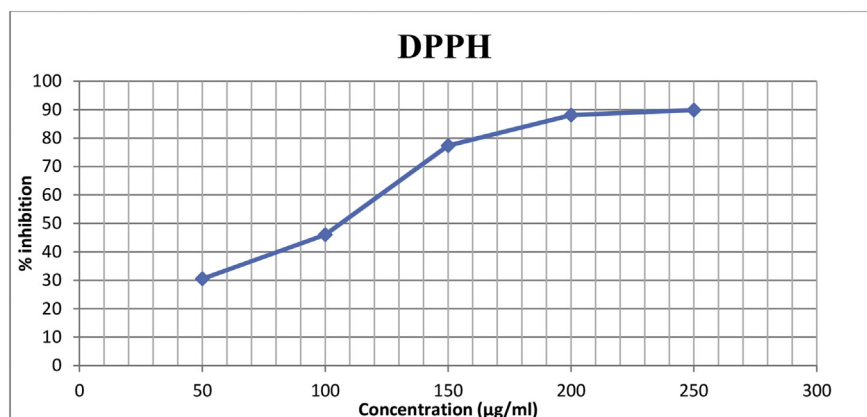


Fig. 5. Effect of ethanolic extract of Cnl on free radical generated in DPPH assay.

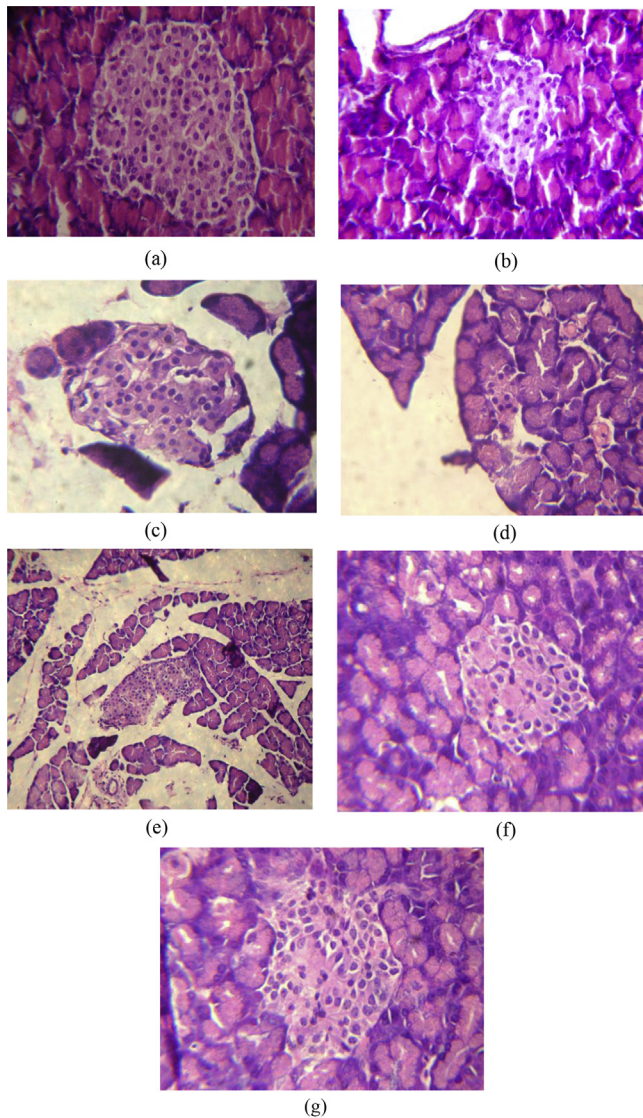


Fig. 6. Representative photomicrographs of haematoxylin and eosin (H and E, 400 \times) stained sections of pancreas. Pancreatic tissue shows atrophy in group II (b) when compared to Group I (a). Treatment groups including group III, IV, V, VI and VII showed reduced damage to pancreas.

analysis of pancreatic tissue showed an improvement in the pancreatic tissue integrity and altered architecture of the tissue upon treatment with CnI extract alone and in combination with metformin.

5. Conclusion

In conclusion, the present investigation suggests that CnI extract possesses phytonutrients with antidiabetic properties. The data supports the antihyperglycemic activity of combination consisting of CnI extract and metformin in STZ induced diabetic rats. Particularly, the study also explains that the combination therapy produces a greater antidiabetic effect than that of the extract alone which could be an effective and alternate way to reduce the dose of synthetic drug and thereby its side effects. Future work needs to be done in the direction of

the use of CnI extract and metformin so as to elucidate the molecular mechanisms which are involved in the use of drug-herbs for prevention of this metabolic syndrome.

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Conflict of interest

None.

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