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In-vivo anti-hyperglycemic effect of herbal extracts *Tribulus terrestris* (L) and *Curcuma amada* (R) on streptozotocin-induced diabetic rats and its associated histopathological studies

Kumaravel Kaliaperumal [a], Bilal Ahmad Bhat [b], Kumaran Subramanian [c], Thiruchelvi Ramakrishnan [d], Elanchezhiyan Chakravarthy [b], Lamya Ahmed Al-Keridis [e], Irfan Ahmad [f], Nadiyah M. Alabdallah [g,h], Mohd Saeed [i], Rohini Karunakaran [j,k,*]

[a] *Unit of Biomaterials Research, Department of Orthodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India*
[b] *Unit of Ethnopharmacology, Department of Zoology, Annamalai University, Chidambaram, Tamil Nadu, India*
[c] *P.G.Research Department of Microbiology, Sri Sankara Arts and Science College (Autonomous), Enathur, Kanchipuram, Tamil Nadu, India*
[d] *Aman Aqua Bio Chemical Free Plant Activator Manufacturer, Rameshwaram, Tamil Nadu, India*
[e] *Department of Biology, Faculty of Science, Princess Nourah Bint Abdulrahman University, P.O.Box 84428, Riyadh, 11671, Saudi Arabia*
[f] *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia*
[g] *Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, P.O.Box 1982, 31441, Dammam, Saudi Arabia*
[h] *Basic and Applied Scientific Research Centre, Imam Abdulrahman Bin Faisal University, P.O.Box 1982, 31441, Dammam, Saudi Arabia*
[i] *Department of Biology, College of Sciences, University of Hail, Hail, Saudi Arabia*
[j] *Department of Computational Biology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India*
[k] *Unit of Biochemistry, Faculty of Medicine, AIMST University, Semeling, Bedong, Malaysia*

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ABSTRACT

Dia/betes is a serious health concern in many countries with high blood glucose, obesity, and multiple organ failures in late stages. Treating diabetes with effective drugs is still a challenging issue since most of the available diabetic drugs are not effective in combating diabetes, especially in secondary disease complications like obesity, retinopathy, and nephropathy associated with diabetes. Hence search for effective antidiabetic medication, especially from natural sources is mandatory with no adverse side effects. In the present study, a combined herbal aqueous extract of *Tribulus terrestris* and *Curcuma amada* was administered to diabetic-induced rats for 37 days. During experimentation, the mean blood glucose level was estimated and at the end of the experiment on the 37th day, the animal was sacrificed and observed for weight gain, plasma insulin, glycogen, glycated hemoglobin, urea, and creatinine level. The results revealed that TT and CA extract-treated diabetic groups significantly lowered the mean blood glucose level followed by increased glycogen and insulin level. Urea, creatinine, and HbA1c levels were considerably reduced in TT and CA-treated diabetic animals as compared to that of antidiabetic drug Glibenclamide-treated groups. TT and CA-treated diabetic animals showed considerable net body weight gain at the end of the experimental day. A concluding remark of the study shows that

* Corresponding author. Unit of Biochemistry, Faculty of Medicine, AIMST University, Semeling, Bedong, Malaysia
E-mail address: rohini@aimst.edu.my (R. Karunakaran).

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TT and CA herbal extract is effective against diabetes and it can be considered as an antidiabetic agent in ayurvedic medicine practice.

1. Introduction

Diabetes is a metabolic malfunction and disease type characterized by high blood glucose levels due to low insulin level secretion or insulin uptake by cells because of resistance mechanisms [1]. As per the World Health Organization report diabetes will be the top mortality and morbidity-causing disease type worldwide in 2030 [2]. Diabetes mellitus leads to secondary complications like diabetic retinopathy, diabetic nephropathy, and gas gangrene formations [3]. Diabetes-associated hyperlipidemia and high blood pressure becomes a disease cluster that is very complicated to treat with single-drug therapy. This makes physicians opt for multiple drug therapy to treat diabetes which eventually causes adverse side effects in diabetic patients [4,5]. Hence search for drugs with no adverse side effects and effective in treating diabetic-related complications is necessary these days. Type 1 Diabetes is a long-term, autoimmune condition in which the pancreas is unable to produce insulin, a hormone that helps regulate blood glucose levels [6]. The pancreas produces less insulin over time, eventually ceasing to produce any at all. Type 1 diabetes is a condition in which the immune system malfunctions, destroying beta cells, which are responsible for producing insulin. This damage can be caused by infection or other external factors. Insulin is necessary for the survival of individuals with this condition, as high blood glucose levels can cause long-term damage to internal organs [7].

The Global Burden of Diseases, Injuries and Risk Factors study (GBD) reports states that nearly 460 million people survived with diabetes in 2019 worldwide which is the eighth largest disease causing mortality among the population [8,9]. International Diabetes Federation (IDF) reports that in 2021 nearly 537 million people had diabetes which incur about 966US$ health expenditure worldwide and it may increase up to 1054 US$ by the end of 2045 [10]. In 2020 Lancet Commission on diabetes reported that 80 % of the diabetic population is from low-income and middle-income countries (LMICs) [8].

Traditional medicinal plants have been used from time immemorial for treating diabetes in many countries like China, India, Southeast Asia, and African countries [11,12]. Plant-based medicine acts not only as a pharmacological approach but also considered a plant-based diet as veganism to treat many human ailments [11–13]. In ethnopharmacological evidence, so far more than 800 plants have been reported to possess hypoglycemic effects and many of the plants are highly significant with antidiabetic properties which make them scrutinized for antidiabetic molecule isolation [14]. A lot of scientific evidence based on complementary and alternative medicines to treat diabetes mellitus is reported especially with individual bioactive compounds isolated, characterized, and targeted against diabetes [15,16]. Traditional medicinal plant extracts and their perspective individual bioactive metabolites were found to reduce the blood glucose level by shutting the metabolism of the AMPK/PI3K/AKT signaling pathway in pancreatic and muscle cells [17–21].

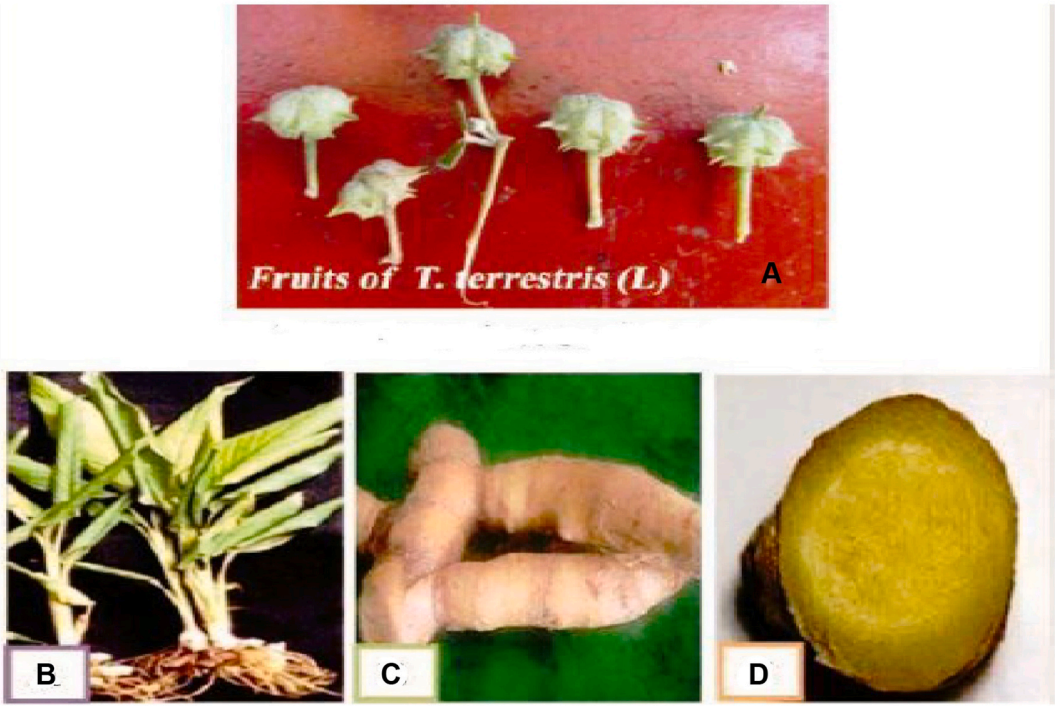

Fig. 1. Herbal samples. A: Fruits of *Tribulus terrestris*. B: *Curcuma amada* plant. C: Rizhome of *Curcuma amada*. D: Cut section of *Curcuma amada*.

Tribulus terrestris (TT) is an Indian herb used in Ayurvedic and folkloric medicine long back to treat several diseases. TT is being used as an aphrodisiac, anti-urolithiasis, diuretic, urinary disinfectant, and antihypertensive [22–25]. TT is used to treat impotence and sexual dysfunction problems in Chinese traditional medicine and Bulgarian folkloric [26]. In the same *Curcuma amada* (CA) a rhizome herb is used as a spice in Indian cuisine which acts as an appetite inducer and astringent [27]. Besides its food properties, it has immense pharmacological value in terms of purgative [28], anti-tussive [29], and blood pressure regulation [30]. Phytochemicals like Curcumin isolated from *Curcuma* are a validated antioxidant drug moiety that is being used commercially [31,32]. In the present study, a combined formulated herbal preparation of TT and CA was prepared to test its antidiabetic effect against type I diabetes through an In-vivo experimental animal model by studying blood glucose level, insulin, C-peptide, histopathological analysis, and other biochemical parameters associated with diabetes.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin (STZ) (99.9 %) pure was obtained from Sigma Aldrich, India. All the necessary chemicals required for biochemical estimation and histopathological fixatives were procured from Hi-Media Labs (India). All the chemicals and reagents used are of analytical grade (A.R). Milli-Q water was used for reagent preparations.

2.2. Sample collection

The herbal samples of *Tribulus terrestris* (TT) fruits and *Curcuma amada* (CA) rhizome were purchased from the weekly herbal market Vadalur township (11.5688° N, 79.5545° E), Tamil Nadu, India (Fig. 1A–D). The collected samples were identified by the Department of Botany, at Annamalai University. A voucher specimen of TT and CA was deposited in the herbarium of the Department of Botany and voucher specimen ID for TT (AUB: 54124) and CA (AUB: 87451) was generated for future reference.

2.3. Preparation of herbal extract

The extraction methodology of the plant materials used in the present study was confined and adopted based on [33,34] with slight modifications. Herbal samples of TT and CA were shade-dried for a month time. Around 4 kg of each TT and CA were extracted with aqueous (Milli-Q) (w:v) three times. The aqueous extract was then filtered and lyophilized (Esquire Biotech, India) for drying. The obtained dry aqueous extract powder was used for In-vivo animal experimentation by dissolving in normal saline. The extraction methodology was optimized based on the nature of phytochemical constituents present in the *Curcuma amada* [30,32] and *Tribulus terrestris* [35,36] which is validated through HPLC and GC-MS quantification.

2.4. Animal experimentation

Albino Wistar rats male (140 ± 0.6 g) were used for the present study. Animal groups were categorized into 6 groups consisting of 6 animals each. Animals were caged in polypropylene cages housed in ventilated animal houses. The temperature was maintained at (30 °C ± 0.4) for less than 24 h of dark and light cycle. Constant water supply was provided to animals using animal feed bottles and animals were fed with a Standard pellet chow feed diet (Biotechnika Labs, Banglore, India). The dose fixation of herbal extracts was done based on the previous experimental research findings [37–40].

Group I: Normal vehicle control.
Group II: Diabetic control.
Group III: Diabetic + TT extract treated (100 mg/kg body weight).
Group IV: Diabetic + CA extract treated (100 mg/kg body weight).
Group V: Diabetic + CA extract (50 mg/kg b.w) + TT extract (50 mg/kg b.w).
Group VI: Diabetic + Glibenclamide (900 μg/kg b.w).

2.5. Diabetic induction

Diabetes was induced in animal models using Streptozotocin following the methodology of [41,42]. In brief 32-h, fastened rats were induced diabetes by intraperitoneal injection of Streptozotocin (60 mg/kg b.w prepared in 0.1 M Normal saline). After a week time, the blood glucose level was checked using a blood glucose meter (Accucheck, Inc.) by pricking ear lobes. Rats with blood glucose levels of 260 mg/dL and above are a hallmark of diabetes onset. The experimental setup was continued for 37 days. At the end of the 37th day animals were sacrificed by standard euthanasia procedure and the blood, and tissue samples were collected, processed, and stored at −80 °C until use [39,40]. All the animal experiments were carried out after obtaining the standard Institutional Animal Ethical Committee (CPCSEA/IAEC/AU:234/2018).

2.6. Body weight estimation and blood glucose

The body weight of the animals before and at the end of the experiment is tabulated and recorded as a standard error mean value

daily [32]. Blood glucose was estimated by following the methodology of [33] with slight modifications. In brief, the daily blood glucose before and after the experiment was assessed through a glucometer, and the mean value was tabulated. At the end of the 36th day, the animals were sacrificed and the blood was collected in a heparinized tube which was then subjected to glucose estimation biochemically [43,44].

2.7. Estimation of glycosylated hemoglobin (HbA_1C)

Glycosylated hemoglobin in the blood was estimated by the method of [45] with slight modification. Glycated hemoglobin in the blood was quantified using a commercial Rat HbA1c/glycated hemoglobin ready-to-use kit (Cat. No: MBS2033689, My Bioresource Inc, USA). The amount of HbA1c present in the control and treated and diabetic groups was expressed in the unit of mg/g of hemoglobin.

2.8. Hepatic glycogen estimation

The hepatic glycogen was estimated by the method of [46]. The glycogen level in the experimental group rats was assessed using the standard rat glycogen estimation readymade kit (Cat. No: MBS729293, My Bioresources Inc, USA). The concentration of glycogen level in the experimental groups was expressed as mg/g tissue.

2.9. Plasma insulin and C-peptide estimation

The plasma insulin and C-peptide level in the experimental animal group was quantified through Radio labeled ELISA method (Enzyme-Linked Immunosorbent Assay) standard rat insulin estimation Kit (My Bioresources Inc, USA) [40,47]. The Insulin level and the value were expressed as μU/mL of plasma and C-peptide level was expressed in terms of ng/mL of plasma.

2.10. Urea and creatinine estimation

Urea and creatinine concentration in the blood plasma was estimated by ready-to-use standard Rat Blood Urea Nitrogen (BUN) ELISA kit (Cat.No: MBS2611086) and Creatinine (Cat.No: MBS9300729, My Bioresources Inc, USA). The value was expressed as mg/dL of plasma [45].

2.11. Histopathology analysis

Pancreas, liver and kidney tissues from the experimental groups were dissected at the end of the 37th day of the experiment. Tissues were washed with ice-cold water extensively and fixed in 10 % formalin followed by paraffin wax embedding for microtome sectioning (5 μm) thickness. The microtome-sectioned tissue samples are then subjected to staining procedures like eosin and hemotoxylin for microscopic observation [44].

2.12. Statistical analysis

The data were obtained in triplicate assay and expressed in standard error mean. One-way ANOVA was performed followed by a student t-test and Post hoc multiple comparison test to validate the data as statistically significant with a p-value of <0.05. All the statistical analysis was performed using SPSS.24 version statistical package.

3. Results

3.1. Body weight estimation and blood glucose

The herbal formulation of TT and CA-treated animal groups showed a considerable weight gain increase merely equal to the normal

Table 1
Antidiabetic effect of TT and CA on body weight, plasma glucose, and plasma insulin.

Groups	Body weight (g)		Net Weight gain (g)	Plasma glucose (mg/dL)	Plasma Insulin (μU/mL)	C-peptide (ng/mL)
	Initial	Final				
NC	157.98 ± 12.18	193.98 ± 8.72	36 ± 8.7	84.69 ± 8.9	14.09 ± 1.11	0.84 ± 0.15
DC	196.26 ± 14.15	151.99 ± 12.17	−45 ± 12.17	324.5 ± 15.9	7.49 ± 0.3	0.39 ± 0.17
D + TT	160 ± 13.28*	180.66 ± 11.43*	20.66 ± 11.43	205.5 ± 11.5	9.95 ± 0.4	0.61 ± 1.6
D + CA	154.99 ± 12.17	177 ± 13.28	22.01 ± 13.28	199.79 ± 10.4	10.34 ± 1.31	0.55 ± 0.67
D + TT + CA	157.63 ± 6.87*	180 ± 10.44*	22.37 ± 10.44*	139.8 ± 9.2*	12.51 ± 0.94*	0.74 ± 1.38*
D + GLCD	160 ± 11.06	187.16 ± 11.43*	27.16 ± 11.43*	121.18 ± 7.97*	13.1 ± 1.51*	0.80 ± 1.47*

Values are given as mean ± SD (n = 6 rats). Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$.

control groups. The diabetic animal groups exhibit drastic weight loss which is a serious diabetic ailment issue in all diabetic patients. Following the administration of herbal extracts TT, CA, and TT + CA the diabetic animals showed a recovery pattern in body weight in terms of net weight gain as significant to that of Glibenclamide antidiabetic drug-treated group. In the current experiment, the net body weight before and after the experiment study in the control group was observed with (157.98 ± 12.18g) and (193.98 ± 8.72g), and the average weight gain was recorded as 36g. The growth was slightly increased in control treated with TT and CA. The initial and final growth of TT treated group was (164 ± 10.98g) and (199.33 ± 12.49g), respectively. The average growth was (35.33g). Similarly, the initial and final growth of CA was (165.44 ± 12.49g) and (199.93 ± 4.65g), respectively and the average growth recorded was 34.29g (Table 1).

The blood glucose level in the diabetic group was recorded as high (324.5 ± 6.9 mg/dL) when compared to the normal control group (84.69 ± 8.2 mg/dL). There is a significant reduction in the blood glucose level of diabetic animals treated with the herbal extract of TT, CA, and TT + CA as equal to that of the antidiabetic drug glibenclamide treated group as (205.5 ± 11.5 mg/dL), (199.79 ± 10.4 mg/dL) (139.80 ± 9.20 mg/dL) and (121.18 ± 7.97 mg/dL). The combined herbal formulation of (TT + CA) treated diabetic groups considerably reduced the blood glucose level as significant to that of the diabetic drug glibenclamide treated group (Table 1). The plasma insulin and C- peptide level was drastically low in a diabetic individual with 7.49 ± 0.3 (μU/mL) and 0.39 ± 0.17 (ng/mL) which is due to the destruction of beta cells in the pancreas by STZ treatment. Followed by herbal extract administration the plasma insulin and C-peptide levels recovered to a considerable level significant to that of the Glibenclamide drug-treated group (Table 1).

3.2. Estimation of glycosylated hemoglobin (HbA_1C), glycogen, plasma insulin, urea, and creatinine

The glycated (HbA_1C) concentration in diabetic groups was (8.04 ± 0.57) HbA1c% which is higher than that of normal control (4.01 ± 0.35 HbA1c %). The combined herbal extract of TT and CA-treated diabetic groups showed considerably lesser glycosylated hemoglobin of (5.79 ± 0.5 HbA_1c %) which is significant to that of the drug-treated group (5 ± 0.4) (Table 2). Followed by the HbA_1C, the plasma insulin level is found to be reduced in the diabetic-induced group (7.49 ± 0.3) which is a hallmark of a diabetic condition. TT and CA treatment recovered the insulin secretion in diabetic groups (12.51 ± 0.94) as equal to that of normal (14.09 ± 1.11) and drug-treated (13.1 ± 1.51) (Table 1). Recovery of insulin secretion in diabetic groups after herbal administration indicates that the herbal formulation is a good antidiabetic agent. The liver glycogen level is found to be lower in diabetic rats (23.14 ± 2.14 mg/g) since STZ damages liver tissues. At the same time, combined TT and CA administration recovers the glycogen level to a significant amount of (34.94 ± 4.14 mg/g) which is equally significant to the Glibenclamide treated group (35.54 ± 4.12 mg/g) (Table 2).

The level of urea in diabetic rats is marked up to the level of (30.24 ± 3.13 mg/dL). High urea content in diabetic individuals denotes that frequent urination is a common phenomenon in diabetic patients. At the same time, combined herbal formulation treatment of TT and CA in diabetic groups exhibited (24.14 ± 2.27 mg/dL) urea level. Glibenclamide drug treatment also considerably reduced the urea level up to (21.19 ± 2.12 mg/dL) (Table 2). Creatinine a muscle breakdown product that is excreted through urine is an important factor in diabetes. In the present study, the diabetic animals showed a higher creatinine level of (1.28 ± 0.18 mg/dL) which is a higher level of creatinine concentration when compared to the normal level of (0.49 ± 0.04 mg/dL). Herbal treatment of TT and CA reduced the creatinine level up to 0.77 ± 0.07 mg/dL) which is significant to that Glibenclamide drug-treated group with (0.74 ± 0.06 mg/dL) (Table 2). However, the individual plant extract of TT and CA-treated group showed decreased creatinine, urea, and blood glucose levels, and the combined formulation of TT with CA showed a better antidiabetic effect.

3.3. Histopathology observations

The histopathological analysis of the liver, kidney, and pancreatic tissues was done through microscopic observation. The liver of diabetic and herbal-treated groups exerted significant changes in tissue morphology. The liver histology examination revealed that the control group is characterized by intact hepatocytes and blood vessels. The STZ-induced diabetic groups are characterized by atrophied hepatocytes and lesions in blood vessels and damaged blood sinusoids to a greater extent. At the same time, the herbal extract TT and CA treated group exhibits regenerated liver microstructures from STZ-induced damage both in terms of structure and functional physiology (Fig. 2: A-F).

In the same way, the kidney histology analysis revealed that diabetic kidneys showed damaged nephric duct and glomeruli which are recovered after consecutive plant treatments of TT and CA individually and combined form (Fig. 3:A-F). The pancreatic tissue exhibited similar atrophied lesions in diabetic groups with damaged beta cells and adjacent matrix tissues (Fig. 4:A-F). Plant samples of

Table 2
Antidiabetic effect of TT and CA on Glycosylated Hb, Liver glycogen, Urea and Creatinine.

Groups	Glycosylated Hb (HbA_1C %)	Liver glycogen (mg/g)	Urea (mg/dL)	Creatinine (mg/dL)
NC	4.01 ± 0.35	40.14 ± 4.14	19.49 ± 2.17	0.49 ± 0.04
DC	8.04 ± 0.57	23.14 ± 2.14	30.24 ± 3.13	1.28 ± 0.18
D + TT	7.23 ± 0.42	30.24 ± 2.14	27.25 ± 2.82	0.89 ± 0.08
D + CA	7.04 ± 0.58	33.24 ± 3.43	26.20 ± 2.52	0.84 ± 0.08
D + TT + CA	5.79 ± 0.5*	34.94 ± 4.14*	24.14 ± 2.27*	0.77 ± 0.07*
D + GLCD	5 ± 0.4	35.54 ± 4.12*	21.19 ± 2.12*	0.7 ± 0.06*

Values are given as mean ± SD (n = 6 rats). Values that do not share a common superscript letter in the same column differ significantly at $p < 0.05$.

TT and CA treatment in diabetic groups immensely recovered the tissue damage with repaired Islets cells and blood vessels. The histopathological observation confirmed that the plant-treated diabetic groups showed significant tissue regeneration in terms of morphology, and physiology when compared to the drug Glibenclamide treated groups.

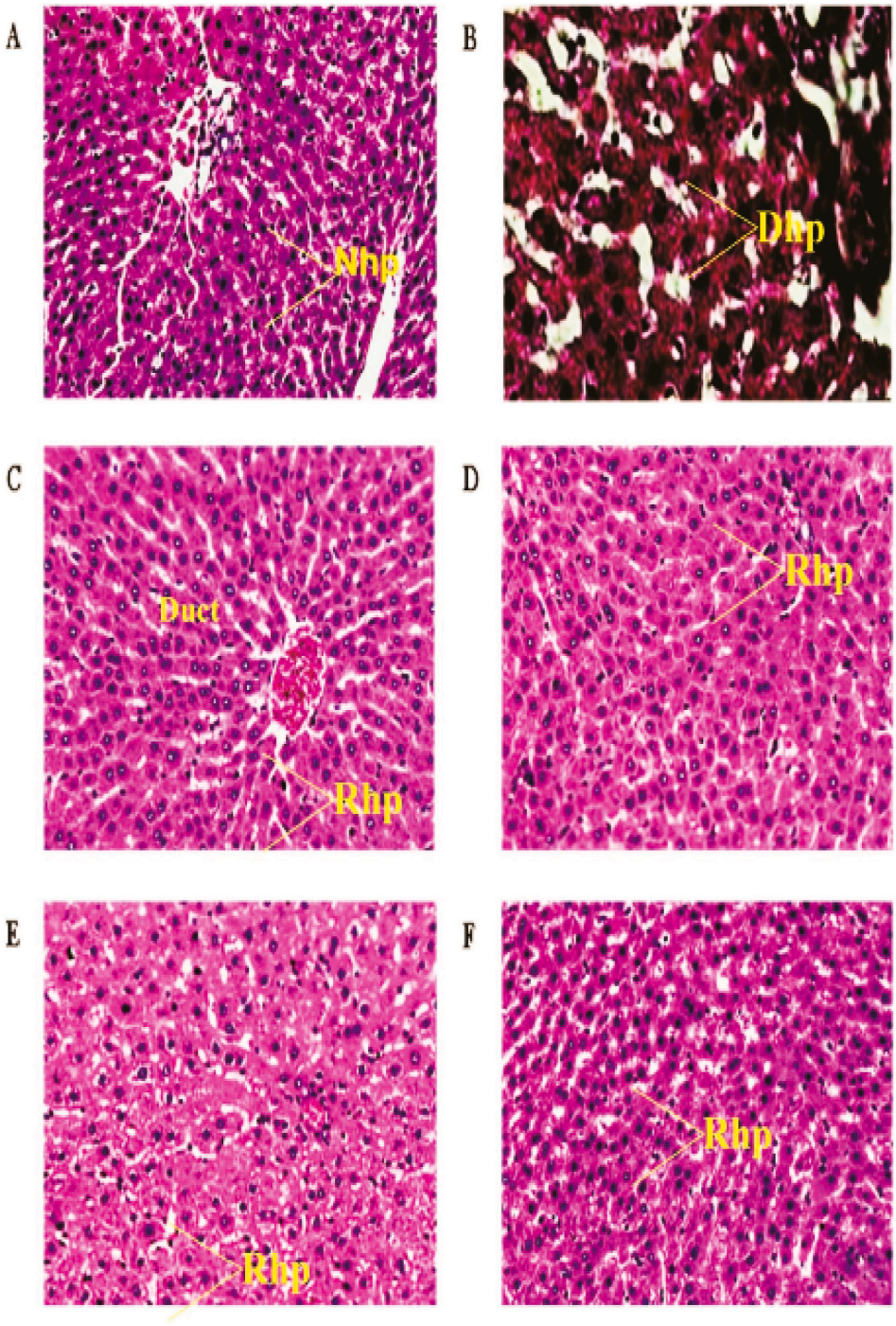

Fig. 2. Histopathology of liver tissue. A- Control liver with intact normal hepatocytes (Nhp), B- Diabetic liver (STZ treated) (Dhp: Damaged hepatocytes), C- *T.terrestris* treated liver (Rhp: Recovered hepatocytes), D- *C.amada* treated liver, E– T + C treated liver (Combination of two extracts), F- Glibenclamide treated liver (Standard Drug).

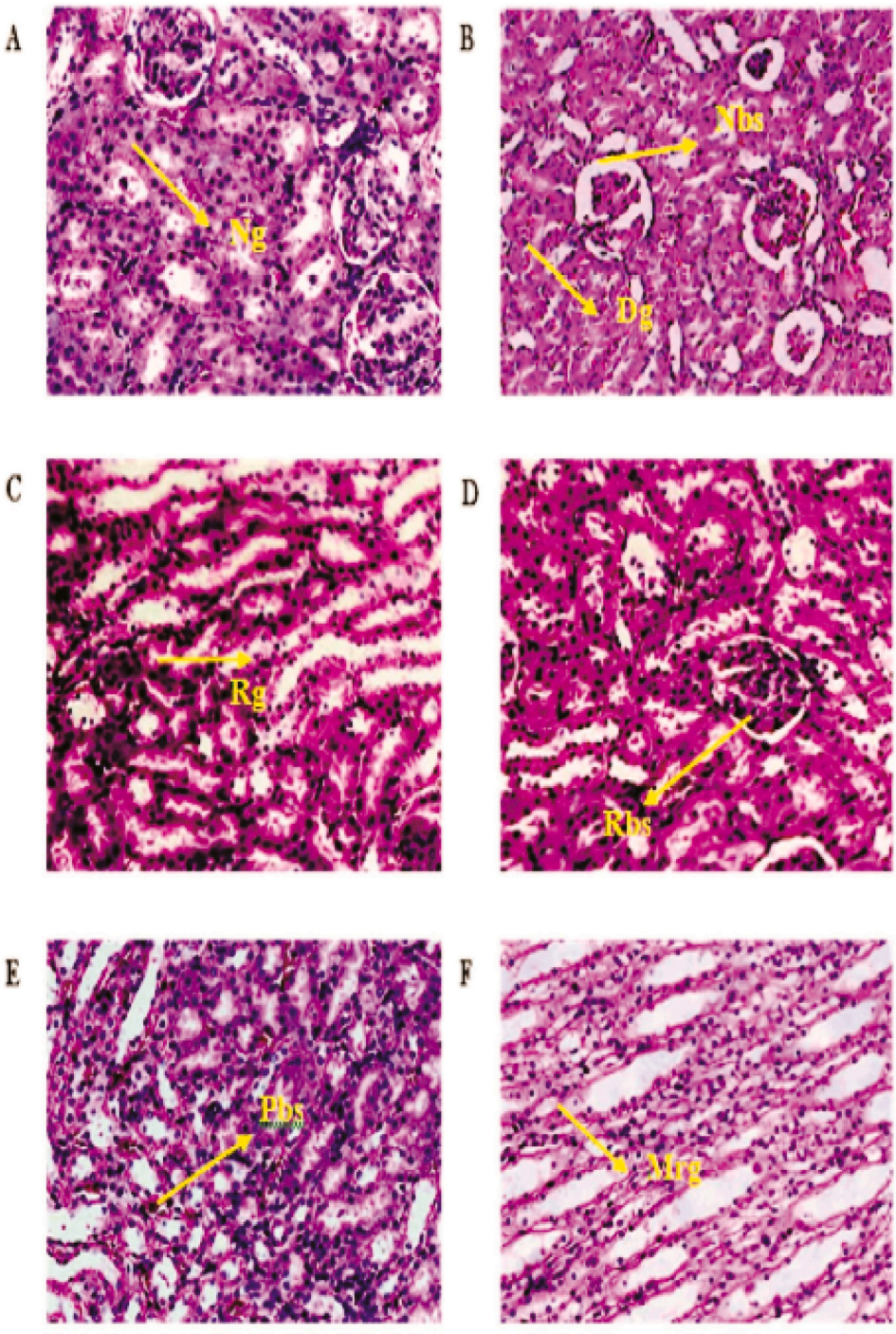

Fig. 3. Histopathology of kidney tissue. A- Control. B- Diabetic (Ns: Necrotized sinusoids; Dg: Damaged glomeruli), C- *T.terrestris* treated Kidney (Rg: Regenerative glomeruli), D- *C.amada* treated Kidney (Rbs: Regenerative blood sinusoids), E– T + C treated Kidney (Pbs: Prominent blood sinusoids), F- Glibenclamide treated Kidney (Mrg: Moderate regenerative glomeruli).

4. Discussion

Diabetes is a serious illness characterized by high blood glucose levels which leads to fatality in many cases if not treated. The available antidiabetic drugs in the market increase drug resistance in diabetic patients as well as bring adverse side effects [48–50]. In recent days many diabetic patients have drug resistance diabetes followed by insulin resistance shown by Type-2 diabetes [51]. Hence

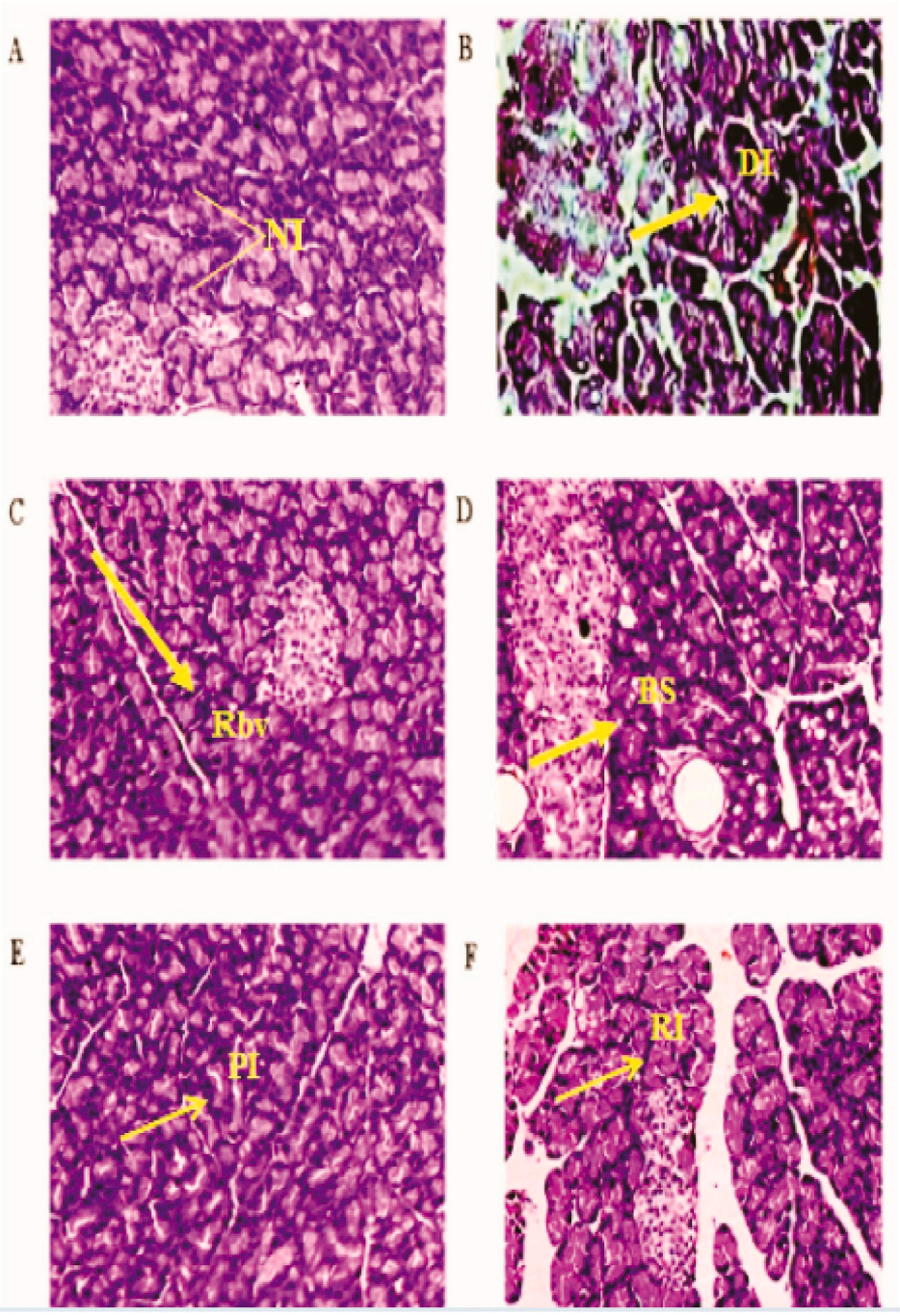

Fig. 4. Histopathology of pancreatic tissue. A- Control (NI: Normal Islets), B- Diabetic (DI: Damaged Islets), C- *T.terrestris* treated (Rbv: Recovered blood vessels), D- *C.amada* treated Pancreas (Blood sinusoids), E− T + C treated Pancreas (PI: Prominent Islets), F- Glibenclamide treated Pancreas (RI: Recovered Islets).

a conventional medication therapy without any adverse side effects followed by effective antidiabetic properties should be explored. Medicinal plants have been used to treat diabetes in Indian Ayurveda and Siddha medicine systems for a long back. In many Asian and African countries, the traditional folkloric medicinal plant-based diabetic cure is put into practice as an effective antidiabetic agent [52,53].

In the present study two traditional medicinal plants namely *T.terrestris* and *C.amada* combined herbal formulation was validated for their antidiabetic efficacy through the in-vivo animal model. The animals were induced to be diabetic using STZ injection. Followed by diabetic induction administration of herbal formulation TT and CA separately and in combined form to diabetic animals showed a

significant decrease in diabetic markers profile in terms of low blood glucose, glycated HbA_1C, urea, and creatinine. The plasma insulin level was found to be increased in diabetic animals treated with TT and CA as significant to that of the diabetic drug Glibenclamide-treated group. TT and CA extract individually as well as combined administration is observed with increased body weight after the experimentation at the end of the 37th day. This shows that TT and CA can also be nutraceutical agents for weight gain practice. It is believed that saponin a phytochemical constituent present in the TT is responsible to exert a hypoglycaemic effect that significantly reduce serum glucose, cholesterol, and antioxidant enzyme superoxide dismutase (SOD) which is previously validated through alloxan-induced diabetic models [54]. Similarly, the CA extract contains major phytochemical constituents like phenols, flavonoids, and alkaloids that seem to possess an antidiabetic effect which is validated through STZ-induced diabetic rat models [55].

TT is used as an anti-diuretic herb in Indian medicinal practice to treat kidney stones. In earlier findings, methanolic TT extract was found to dissolve kidney stones in the Guinea pig model effectively by oral administration [56]. Apart from the antidiuretic properties, the TT plant is proven to be antihelminthic [56], antibacterial [57], antiplasmodial [58], and anti-cancerous [59]. In the same way, *Curcuma amada* a rhizome herb is being used in Indian traditional medicine as an antimicrobial, antioxidant, astringent and blood dilation, and antihypertensive [60–62]. CA was used as food in Indian cuisine as culinary practice in terms of spice and appetite inducer. Due to its immense multipurpose application, many Western countries claimed CA for their geographical and product-based patent rights [63]. The present study is a first-time effort to evaluate the combined herbal extract of TT and CA through the in-vivo antidiabetic model.

The results of the present study are promising enough that the combined TT and CA extract exhibited a significant antidiabetic effect in lower diabetic markers and the histopathological analysis revealed that TT and CA-treated diabetic groups showed considerable tissue recovery from the damage induced by STZ diabetic induction compared to that of Glibenclamide drug. STZ-induced diabetic animals have profound tissue necrosis in the pancreas, liver, and kidney in the form of damaged blood sinusoids, nephric ducts, Islet cells of Langerhans, and hepatocytes. Due to this severe tissue damage insulin production is recorded as low in diabetic rats along with less glycogen level. TT and CA administration leads to tissue regeneration in all the tissues so far analyzed in the present study which are correlated with data of insulin, and glycogen parameters concentrations. This postulates that polyherbal formulation of TT and CA recovers the STZ-induced tissue damage in the pancreas and revives insulin secretion. Many herbal extracts with enriched phytochemicals have been proven to be effective in tissue regeneration in many experimental studies [46]. In the present study, a synergistic effect of phytoconstituents present in the TT and CA extract brings a significant antidiabetic effect in the experimental animal models. In modern Ayurveda and homeopathic traditional medicinal system prescription of two or more groups of antidiabetic herbal formulations is practiced which is believed to collectively decrease the hyperglycaemic level. It is proved that the synergistic effect of a polyherbal combination is safe and non-toxic as well as feasible enough to exert potential antidiabetic action [64].

5. Conclusion

Herbal products and ethnic medicine have been put into practice to cure diabetes from time immemorial. Due to the easy availability and no adverse effects plant-based medicine is widely accepted in many developing countries rather than to adapt with modern medicines. The recent COVID-19 outbreaks worldwide are effectively tackled with traditional plant medicine in India with Kabasura kudeener (Polyherbal drink). In the present study, a combined herbal formulation extract of TT and CA was found to be effective against diabetes by lowering blood glucose, urea, and creatinine and increasing the restoration of insulin and glycogen levels in diabetic animals. TT and CA extract was found to increase the body weight in diabetic animals which shows its future applications in the form of weight gain nutraceutical supplements. As a concluding remark combined herbal formulation of TT and CA extract is found to be an effective antidiabetic principle through in-vivo studies. The prospect of isolating individual active phytochemical constituents from these plant extracts and testing individually on diabetic models both in-vivo and in-vitro models will be promising enough to come up with drug candidates from these plants. Apart from that these combined herbal extracts can also be used as an adjuvant for nutraceutical supplements for weight gain practice in nutrition-deprived lean individuals with further clinical evaluations.

Data availability statement

The raw data of this experimental study is available with the corresponding author and it can be obtained from the corresponding author consent through mail.

Ethics statement

The animal experiments carried out in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of Annamalai University, Tamil Nadu, India under the approval grant number (CPCSEA/IAEC/AU:234/2018).

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CRediT authorship contribution statement

Kumaravel Kaliaperumal: Writing – original draft, Investigation, Conceptualization. **Bilal Ahmad Bhat:** Writing – original draft, Investigation. **Kumaran Subramanian:** Investigation, Formal analysis, Data curation. **Thiruchelvi Ramakrishnan:** Investigation, Formal analysis, Data curation. **Elanchezhiyan Chakravarthy:** Investigation, Formal analysis, Data curation. **Lamya Ahmed Al-Keridis:** Funding acquisition. **Irfan Ahmad:** Funding acquisition. **Nadiyah M. Alabdallah:** Funding acquisition. **Mohd Saeed:** Resources, Funding acquisition. **Rohini Karunakaran:** Validation, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kumaravel Kaliaperumal reports financial support was provided by King Khalid University.

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