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Antidiabetic Effect of Hexane Extract of Costus Speciosus and Metformin in Rats

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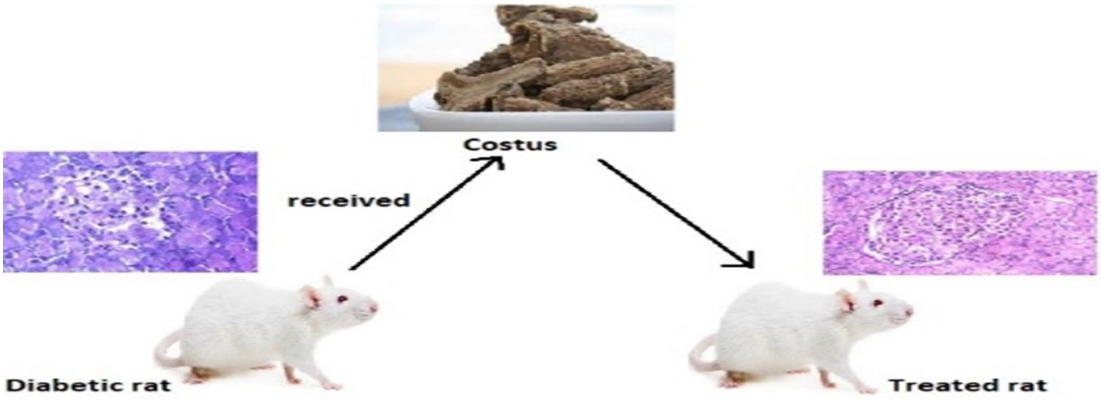
Abstract

Graphical Abstract



The aim of the research was to examine the effects of various antidiabetic agents in albino rats by the selection of two antidiabetic agents, one of which is chemical (metformin) and the other is a natural plant (Costus), and to investigate their possible beneficial effects on diabetes. A total of 36 normal male adults albino rats (Sprague strain) were randomly assigned into six equal groups**.** Three of them were normal rats. The first normal group served as the control group. The second normal group received costus rhizome extract at a dose of 250 mg/ kg B. W. once a day for 30 days. The third normal group received metformin cure at a dose of 500 mg/ kg B. W. once a day for 30 days. The other three groups were diabetic rats that injected STZ (45 mg/ kg B.W. /i.p.). The first diabetic group served as control group. The second diabetic group received costus rhizome extract at a dose of 250 mg/ kg B. W. by oral gavage once a day for 30 days, the third diabetic group received metformin cure at a dose of 500 mg/ kg B. W. by oral gavage once a day for 30 days. Our results showed that metformin and hexane extract of costus sp. treated groups revealed enhancement and improvement of disturbance of liver functions, kidney functions, immunological parameters, antioxidant parameters and histopathology of the pancreas.

Keywords: Diabetes, Costus, Biochemical analysis, Antioxidant, Immunity

1. Introduction

Hyperglycemia is a symptom of diabetes mellitus, which is produced by insulin production, insulin action, or a combination of the two. In its earliest stages, type 2 diabetes, the most common kind, is generally asymptomatic and may go unnoticed for many years. Long-term organ dysfunction, damage, and failure have been related to persistent hyperglycemia, notably in 1the kidneys, eyes, nerves, blood vessels, and heart [1]. Undiagnosed type 2 diabetes increases the risk of stroke, peripheral vascular disease, coronary heart

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disease, and compared to persons who do not have diabetes. Dyslipidemia, hypertension, and obesity are also more common in them. In certain situations, diabetes screening may be advantageous since early identification and treatment can lessen the impact of diabetes and its consequences [2].

An autoimmune attack on the pancreatic islet beta cells causes type 1 diabetes. Insulin deficiency leads hyperglycemia and thus the risk of ketosis when around 80% of beta cells are damaged or destroyed. Gestational diabetes is a kind of diabetes that occurs only during pregnancy and is a precursor to type 2 diabetes later in life [3] .

Over 13.000 plants have been studied over the previous five years. Traditional medicine, which is largely based on plant medicine, is used by 80% of the world's population for their primary healthcare [4]. Herbal remedies were the primary therapy for diabetes and its consequences until the discovery of insulin and other diabetes-controlling drugs [5]. For the treatment of type 2 diabetes, biguanides, sulphonylureas, and thiazolidinediones were available, and they had been proved to be effective hypoglycemic agents. They do, however, have certain negative effects. As a result, numerous laboratories throughout the world are searching for novel medicines with minimal side effects [4] .

Costus specious is one of the most powerful traditional Islamic medicinal herbs. It is verified in the authentic Hadith found in Sunan Abi Dawud, the Book of Medicine (Kitab Al Tibb), in which Umm Qasis, Mihsan's daughter, says: I took my son before Allah's Messenger when I was compressing his uvula for swelling. He said, "Why do you torture your children by squeezing for a uvula swelling?" Apply this Indian aloes wood (costus), which includes seven types of treatments, one of which is a pleurisy cure. Specifically, C. specious was recommended as a treatment for pharyngitis and tonsillitis in children, pleurisy, and as an antidote for snake venom in prophetic medicine [5].

The Zingiberaceae family includes costus specious, sometimes known as the "insulin plant". It is well-known for its anti-diabetic properties and is used as a traditional dietary supplement in the treatment of diabetes in Southern India [6]. The rhizome hexane extract possesses hypolipidemic and anti-hyperglycemic properties, reverses diabetes and its complications, improves hepatic antioxidant enzyme activity, affects monoamine oxidase activity and neurotransmitters, possesses antipyretic and anti-inflammatory properties, and demonstrates significant hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity [7] .

The aim of this study was to compare the effects of hexane extract of costus specious and the effects of metformin on streptozotocin-induced diabetic rats by evaluating gas chromatography / mass spectroscopy analysis, serum biochemical analysis, serum antioxidant analysis, serum immunology analysis, and histopathology examination

2. Materials and Methods

2.1. Experimental animals

A total of 36 male albino rats weighing between 150 and 230 grams were used. The animals used in this investigation were from the Suez Canal University Pharmacy Faculty's laboratory animal home. The animals were given a week to acclimate to the laboratory animal home. The rats were divided into six groups, each with six rats, and housed in a cages with a 12-hour light-dark cycle at a constant temperature of 30 ±2 C. For one week (adaptation period), rats were maintained in normal, healthy environments and provided with a commercially balanced diet. The commercial balanced diet consisted of 4.12% casein, corn 4%, soya 8.8%, vitamin mixture 1%, salt mixture 0.5%, mineral 1% and bran 14.34%. Water was provided ad libitum [8].

2.2. Drugs and chemicals

Streptozotocin was purchased from MP Biomedical Company (Solon, Ohio 44139) (CAS 18883-66-4 batch 100557). Cidophage (Metformin HCL) was purchased from a local pharmacy. It was provided as a tablet by CID, Egypt. Costus was purchased from a local market as bark, then grounded and made into a powder. Special kits for biochemical parameters (AST, serum total protein,

albumin, fasting blood glucose, cholesterol low-density lipoprotein (LDL), triglyceride, high density lipoprotein (HDL) and creatinine were determined in the serum using (Spinreact, Spain), urea (Diamond Diagnostic), ALT (AGD clinipak) commercial kits according to manufacturer protocol. Insulin is calculated by the ALPCO Rat Insulin ELISA is intended for quantifying insulin in rat serum. Alpha glucosidase inhibitory is estimated by a colorimetric method using the BioVision kit. TNF-α (tumor necrotic factor-alpha), IL-6 (interleukin 6), and CRP (C-reactive protein) levels in serum have been determined using the ELISA method and a commercially available kits from (Kamiya Biomedical Company for TNF-α and IL-6 Immunoassay, Genesis Lab Diagnostic Reagent for CRP). **S**uperoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), and malondialdehyde (MDA) are estimated by a colorimetric method using a biodiagnostic kits.

2.3. Costus extract preparation

One kilogramme of costus rhizome powder was soaked for 72 hours in 3 litres of hexane (1:3 ratio) with occasional shaking. The filtrate was filtered with a Bugner funnel and concentrated at 40^0C using a vacuum rotary evaporator until it was dry. A weighed amount of the extract has been suspended in a 0.5 percent aqueous CMC solution (Carboxy Methyl Cellulose) [4]**.**

2.4. Induction of diabetes mellitus

Diabetes mellitus has been generated via a single intraperitoneal injection of freshly synthesized STZ (45 mg/kg B.W.) in 0.1M citrate buffer (pH 4.5) in a volume of 0.9 ml/kg B.W. [8]. Diabetes has been stabilized in these STZ-treated animals after 7 days. Citrate buffer was administered to the animals used as a control (pH 4.5). After seven days, blood was collected from each rat and its serum glucose level was determined. Diabetes rats were included in the study if their fasting serum glucose level exceeded 300 mg/dl.

2.5. Experimental design

Group I: Healthy control group were fed a commercially balanced diet for the whole experiment period. **Group II**: The diabetic control rats that were injected with STZ (45 mg/kg B.W., i.p.) after a weak of STZ injection and served as a control group over the experimental period. [9] **Group III**: Normal rats were fed a commercially balanced diet and received costus rhizome extract at a dose of 250 mg/ kg B. W. by oral gavage once a day over the experimental period [10]. **Group IV**: Diabetic rats that were injected with STZ (45 mg/kg B.W.., i.p.) after a weak of STZ injection and received costus rhizome extract at a dose of 250 mg/kg B.W. by oral gavage once a day over the experimental period. **Group V**: Normal rats were fed a commercially balanced diet and received metformin cure at a dose of 500 mg/ kg B. W. by oral gavage once a day over the the experimental period. **Group VI**: Diabetic rats that have been injected with STZ (45 mg/kg B.W./i.p.) after a weak of STZ injection and received metformin cure at a dose of 500 mg/kg B.W. by oral gavage once a day over the experimental period. At the end of the experimental periods (30 days) rats were sacrificed under diethyl ether anesthesia [11].

2.6. Identification of chemical composition of the extract:

The chemicals were tentatively identified by comparing the mass spectra and retention times of the compounds to the NIST and WILLY library data from the GC/MS instrument [12].

2.7. Serum biochemical analysis: -

The serum level of ALT, AST, and creatinine was determined calorimetrically according to (Murray R., 1984) [13, 14]. Total protein content in serum was determined calorimetrically according to (Koller , 1984) [15] . Albumin in serum was determined calorimetrically according to (Gendler , 1984) [16]. In order to measure serum globulin, total serum albumin was subtracted from total serum protein (Coles, 1974) [17]. Fasting blood glucose in serum was determined calorimetrically according to (Kaplan , 1984) [18]. Fasting insulin in serum was determined calorimetrically according to [19]**.** Alpha glucosidase inhibitory in serum was determined calorimetrically according to (Pistia-Brueggeman, et al., 2001) [20]. Total

cholesterol, HDL-C, and LDL-C in serum was determined calorimetrically according to (Naito H.K., 1984) [21]. Triglycerides in serum was determined calorimetrically according to (Buccolo G et al., 1973) [22]**.** Serum urea level (mg/dl) was estimated calorimetrically according to (Kaplan A., 1984) [23] **.**

2.8. Immunological parameters analysis: -

TNF-α was determined according to (Beutler et al., 1985) [24] . IL-6 was determined from undiluted serum samples according to (Wong et al., 1988) [25]**.** CRP was determined according to (Anderson et al, 1950 & Fischer et al., 1976) [26, 27] **.**

2.9. Antioxidant parameter analysis: -

CAT was determined according to (Aebi et al., 1984) [28]**.** GP_X was determined according to (Hemmadi et al., 2016) [29]**.** MDA was determined according to (Satoh et al., 1978) & (Ohkawa et al., 1979) [30, 31]**. SO**D was determined according to (Senthilkumar et al., 2021) [32] **.**

2.10. Histopathological examination: -

The pancreatic samples were embedded in paraffin after being treated with 10% formalin. Sections of 3μm thickness were presented from each block, mounted on glass slides, stained with hematoxylin and eosin (H&E), and inspected by an independent pathologist.

2.11. Statistical Analysis:

All tested groups in the present study have been assessed utilizing One-Way Analysis of Variance (ANOVA) [33]. Means separations have been done by Duncan's Multiple Range test. These data have been analyzed by SPSS version 20 for Windows. Results can be considered significant when the probability level of 0.05 ($P \leq 0.05$) [34].

3. Results

3.1. Identification of chemical composition of the extract:

The sample GC/MS analysis comprises of fifty chemicals. The discovered chemicals have a total peak area of 100%. The major compounds are 2-Cyclopentene-1-methanol (40.68%), eremanthin (22.32%), costunolide (8.32%), and essential oil (13.88%), which represent (85.2%) of the total peak areas.

3.2. Effect of metformin and costus extract on body weight after 30 days: -

Significant differences have been observed between both the experimental group and the normal control group ($P \leq 0.05$) drop in body weight in the diabetic control group, but no significant ($P \leq 0.05$) difference in body weight between the other experimental groups Table 1.

3.3. The effect of metformin and costus on biochemical Parameters analysis after 30 days:

In comparison to the standard control group, there was a substantial ($P \leq 0.05$) rise in ALT, AST, FBG, - Glucosidase inhibitory, TG, LDL-C, TC, creatinine levels and urea in the diabetic control group. When compared to the normal control group, the diabetic control group had a substantial ($P \leq 0.05$) decline in TP, ALB, GLU, insulin, and HDL-C levels. When compared to the diabetes control group, there was a substantial ($P \leq 0.05$) drop in ALT, AST, FBG, - Glucosidase inhibitory, TG, TC, LDL-C, urea, and creatinine levels in the diabetic groups treated with costus hexane extract and metformin. When compared to the diabetic control group, there was a substantial ($P \leq 0.05$) rise in TP, ALB, GLU, insulin, and HDL-C levels in the diabetic groups treated with costus hexane extract and metformin. There was no statistically significant ($P \leq 0.05$) change in ALT, AST, TP, ALB, GLU, insulin, TC, HDL-C, LDL-C, urea, and creatinine levels between the diabetic groups treated with costus hexane extract and metformin. Although the diabetes group given metformin had a greater drop in insulin levels than the diabetic group given costus hexane extract, the diabetic group given costus hexane extract had a greater fall in - Glucosidase inhibitory, TG levels than the diabetic group given metformin. In all biochemical parameters analyzed, there was no significant ($P \leq 0.05$) difference between the normal control group, the normal group treated with costus hexane extract, and the normal group treated with metformin Table 1**.**

3.4. Effect of metformin and costus on immunological parameters analysis after 30 days: -

Furthermore, as compared to the normal control group, there was a substantial (P≤ 0.05) rise in IL-6, TNF-α, and CRP levels in the diabetic control group. We discovered no significant (P ≤0.05) difference in IL-6, TNF-α, and CRP levels when we compared the normal control group, the normal group treated with costus hexane extract, and the normal group treated with metformin. When compared to the diabetic control group, there was a significant (P ≤0.05) decrease in IL-6, TNF-α, and CRP levels in the diabetic group treated with costus hexane extract and the diabetic group treated with metformin. There was a highly significant (P≤ 0.05) decrease in IL-6, TNF-α levels in the diabetic group treated with costus hexane extract when compared to the diabetic group treated with metformin Table 2.

3.5. Effect of metformin and costus on antioxidant parameters analysis after 30 days: -

Comparing the diabetic control group to the normal control group, CAT, SOD, and GPX levels decreased significantly (P ≤0.05). In contrast, there was a statistically significant (P ≤ 0.05) rise in MDA levels in the diabetes control group compared to the normal control group. There was no significant (P ≤ 0.05) difference in CAT, SOD, GP_X, and MDA levels between the diabetic group treated with costus hexane extract, the diabetic group treated with metformin, the normal control group, the normal group treated with costus hexane extract, and the normal group treated with metformin Table 3**.**

3.6. Effect of metformin and costus on histopathological examination of pancreas in all experimental groups:

The islet cells in a normal control rat pancreas are uniform and regular (Black arrow) **(**Figure 1**)**. Whereas the pancreas of STZ-induced diabetic rats in the diabetic control group showed a shrunken islet cell region with significant cell vacuolization, apoptosis (Arrow head), and intra-insular edema (Black arrow), and there were few inflammatory cells (Red arrow**)** (Figure 2**)**. However, the pancreas of normal costus hexane extract treated rats displayed enlarged islet cells and mild intracellular edema (Black arrow) **(**Figure 3**)**, while the pancreas of diabetic costus hexane extract treated rats displayed enlarged islet cells and intra-insular edema with no cell vacuolization or apoptosis (Black arrow) **(**Figure 4**)**. Moreover, the pancreas of normal metformin-treated rats revealed Uniform regular islet cells with mild intra-insular edema (Black arrow) (Figure 5**)**. The pancreas of diabetic metformin-treated rats revealed enlarged islet cells. Intra-insular edema (Black arrow) with mild cell vacuolization (Arrow head) (Figure 6**)**.

4. Discussion

Diabetes has been developed in rats via a single intraperitoneal injection of STZ at a dosage of 45 mg/kg body weight, and the anti-diabetic effects of hexane extract of costus and metformin, as well as their influence on pancreatic histology, were studied. Diabetes is characterized by progressive metabolic dysfunction, decreasing glycemic control, and structural abnormalities in the pancreas, liver, and other organs. [35]

After 30 days of STZ therapy, the STZ-diabetic control group had a substantial loss in body weight, which might be attributed to poor glycemic control and hence increased protein catabolism and muscle atrophy induced by insulin insufficiency. High protein to amino acid conversion rates for glucose production in diabetic rats may contribute to weight loss. Tannin concentrations can further worsen this process [36].

After 30 days of therapy, there was no significant change in body weight between the diabetic groups treated with metformin and the diabetic groups treated with costus hexane extract. These findings suggest that lower serum glucose levels are linked to lower gluconeogenic activity. Which might explain why diabetic rats treated with costus hexane extract and metformin gained weight [37]**.**

The current investigation of blood biochemical markers revealed a significant increase in the diabetes group's ALT and AST levels throughout the trial. This increase could be because of cellular damage in the liver is due to STZ-induced diabetes.

Group 1

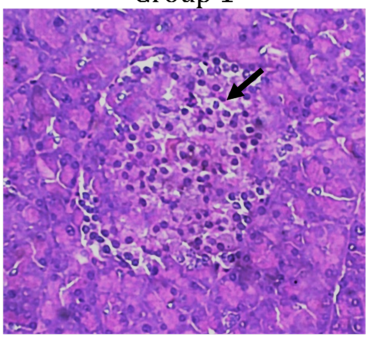

Figure 1: Pancreas of normal rat showing uniform regular islet cells (Black arrow) (H&E, 40x)

Group 2

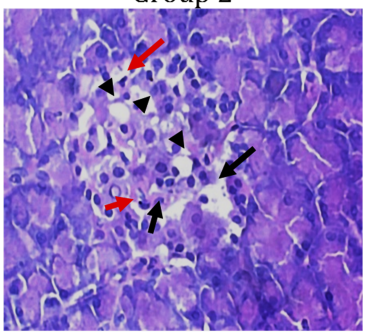

Figure 2: Pancreas of STZ induced diabetic rat showing shrunken islet cell region with significant cell vacuolization with apoptosis (Arrow heads) and intra-insular edema (Black arrows). There are few inflammatory cells (Red arrows) (H&E, 40x)

Group 3

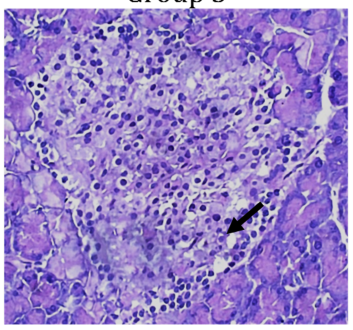

Figure 3: Pancreas of normal rat treated with hexane extract of costus showing enlarged islet cells. Mild intracellular edema (Black arrows) (H&E, 40x)

Group 4

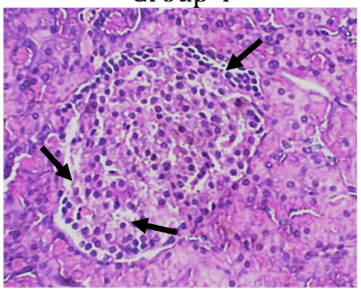

Figure 4:Pancreas of STZ induced diabetic rat treated with hexane extract of costus showing enlarged islet cells. Intra-insular edema (Black arrows) with no cell vacuolization or apoptosis. (H&E, 40x)

Group 5

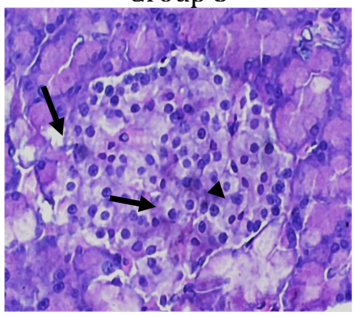

Figure 5: Pancreas of normal rat treated with metformin showing uniform regular islet cells, with mild intra-insular edema (Black arrow) (H&E, 40x)

Group 6

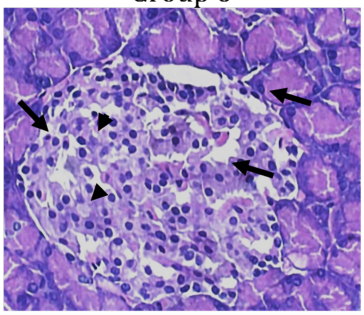

Figure 6: Pancreas of STZ induced diabetic rat treated with metformin showing enlarged islet cells. Intra-insular edema (Black arrows) with mild cell vacuolization (Arrow heads). (H&E, 40x)

Table 1: Biochemical Parameters analysisafter 30 days as measures among studied groups (Means ± SE)

Groups	Normal control	Diabetic control	Normal + Hexane extract	Diabetic + Hexane extract	Normal + Metformin	Diabetic + Metformin
Body weight (g)	283.67±8.74^{a}	166.67±18.56^{c}	239±15.95^{ab}	204 ± 23.86 ^{bc}	253±6.25^{ab}	212±21.66^{bc}
ALT IU/L	40±2.89^{b}	149.33±29.53^{a}	37.1±9.61^{b}	68.47±16.37^{b}	74.67±13.35^{b}	36±7^{b}
AST IU/L	139.33± 27.23^{b}	201±6.66^{a}	142.33±3.28^{b}	158.33±31.22^{ab}	109.67±3.76^{b}	128.67±7.31^{b}
T. PROTEIN g/dl	8.1±0.27^{a}	5.4±0.31^{b}	7.83±0.22^{a}	8.4±0.21^{a}	8.97±0.52^{a}	8.37±0.92^{a}
ALBUMIN g/dl	4.03±0.15^{ab}	2.8±0.12^{c}	4±0.12^{ab}	3.67±0.2^{b}	4.37±0.18^{a}	4.27±0.27^{ab}
Globulin g/dl	4.07±0.12^{ab}	2.6 ± 0.2^{c}	3.83±0.1^{ab}	4.73±0.08^{a}	4.6±0.37^{a}	4.1±0.56^{ab}
FBG mg/dl	120±12.66^{c}	543.33±27.17^{a}	98.33±13.74^{c}	304.67±52.49^{b}	92.67±12.41^{c}	170.67 ± 41.53 ^{c}
Insulin ulU/ml	15.53±0.79^{a}	5.73±0.26^{c}	14.83±0.93^{a}	11.4 ± 0.72^{b}	14.17±0.6 ^{a}	11.4 ± 0.5 ^{b}
ALFA GLUCO %	22.39±0.26^{d}	46.21±0.54^{a}	22.22±0.16^{d}	30.46±0.52 ^{c}	22.52 ± 0.07 ^{d}	35.59 ± 0.39^{b}
TC mg/dl	77.73±1.83^{c}	182.33±6.23^{a}	84.67±2.19^{c}	139 ± 0.58 ^{b}	82.67 ± 4.26 ^{c}	135.33 ± 4.7 ^{b}
TG mg/dl	104.67±2.67^{d}	231±1.16^{a}	99.67±2.9^{d}	123.67 ± 0.33 ^{c}	97.33 ± 5.23 ^{d}	146 ± 6.25 ^{b}
HDL-C mg/dl	44.67±3.33^{c}	25.67±1.2^{a}	41.67±0.33^{cb}	38±1.53^{a}	45.33±2.85^{c}	28.33 ± 0.88^{b}
LDL-C mg/dl	12.13±2.36^{d}	110.47±6.03^{a}	26.13±3.01^{c}	76.27±0.97^{b}	22±1.85 ^{cd}	77.8±4.35^{b}
CREAT mg/dl	0.67±0.03^{b}	1.07±0.09^{a}	0.6±0.06^{b}	0.63±0.09^{b}	0.53±0.09^{b}	0.6±0.06^{b}
UREA mg/dl	28.67±2.01^{bc}	81.67±10.98^{a}	23.4±1.7^{c}	79±19.6^{a}	32.33±3.33^{bc}	58.17±3.87^{ab}

Within the same column, means with different superscripts aresignificantly differ (P ≤ 0.05)

Table 2: Immunology parameters after 30 day as measures among studied groups (Means ± SE)

Parameters Groups	TNF-α pg/ml	IL-6 pg/ml	CRP ng/ml
Normal control	11.42 ± 0.54^{d}	7.61 ± 0.05^{d}	0.26 ± 0.15^{b}
Diabetic control	31.66 ± 0.58^{a}	16.85 ± 0.16^{a}	1.01 ± 0.06^{a}
Normal + Hexane extract	10.84 ± 0.05^{d}	7.53 ± 0.03^{d}	0.13 ± 0.02^{b}
Diabetic + Hexane extract	15.43 ± 0.5^{c}	10.17 ± 0.3^{c}	0.13 ± 0.02^{b}
Normal + Metformin	11.09 ± 0.08^{d}	7.59 ± 0.02^{d}	0.14 ± 0.2^{b}
Diabetic + Metformin	19.58 ± 0.47^{b}	12.6 ± 0.37^{b}	0.18 ± 0.03^{b}

Within the same column, means with different superscripts aresignificantly differ (P ≤ 0.05)

Table 3: Anti-oxidant parameters after 30 days as measures among studied groups (Means ± SE)

Parameters Groups	CAT U/L	SOD U/mL	MDA nmol/mL	GP_X mU/mL
Normal control	92.7 ± 4.38^{b}	42.6 ± 1.27^{b}	72.7 ± 0.84^{b}	48.8 ± 2.19^{b}
Diabetic control	63.3 ± 9.45^{a}	25.67 ± 3.68^{a}	9.34 ± 0.54^{a}	31.13 ± 1.23^{a}
Normal + Hexane extract	94.8 ± 5.43^{b}	40.2 ± 1.1^{b}	$7.43 \pm .052^{b}$	51.83 ± 6.67^{b}
Diabetic + Hexane extract	87.47 ± 3.37^{b}	41.17 ± 2.97^{b}	6.95 ± 0.18^{b}	51.83 ± 1.71^{b}
Normal + Metformin	94.5 ± 4.16^{b}	38.76 ± 1.94^{b}	7.15 ± 0.56^{b}	51.67 ± 1.68^{b}
Diabetic + Metformin	83.3 ± 7.79^{b}	39.47 ± 1.08^{b}	6.59 ± 0.14^{b}	47.37 ± 3.61^{b}

Within the same column, means with different superscripts aresignificantly differ (P ≤ 0.05)

These changes in hepatic function might be due to increased arginase activity and mRNA levels [38].

The administration of a hexane extract of costus sp. improved the levels of ALT and AST as a result of enhanced carbohydrate, lipid, and protein metabolism. The inclusion of flavonoids in the hexane extract of costus sp. may be in charge of the inhibition of ALT and AST from returning to normal levels, since flavonoids have previously been found to be hepatoprotective agents [39, 40].

Metformin reduced liver damage in STZ-diabetic rats administered the drug, as evidenced by a drop in ALT and AST enzyme levels [41].

Metformin is a medication that is used to treat type II diabetes and to enhance liver function in persons who have non-alcoholic fatty liver disease (NAFLD). Metformin stimulates AMP-activated protein kinase (AMPK), a cellular energy sensor that responds to AMP/ATP ratio alterations. AMPK is a mammalian target of rapamycin inhibitor (mTOR). Both mTOR and AMPK can influence cell death [42].

The injection of STZ caused severe liver damage, as evidenced by lower levels of albumin, globulin, and total protein. The decrease in serum total proteins in diabetic rats can be attributed to decrease the uptake the amino acid, significantly lower concentrations of a variety of essential amino acids, an increased conversion rate of glycogenic amino acids to carbon dioxide and water, and a decrease in protein synthesis due to a decrease in mRNA amount and availability. This decline could also be attributed to impaired oxidative phosphorylation mechanisms, which result in a decrease in protein

synthesis, an increase in catabolic processes, and a decrease in protein absorption [43].

The hepatoprotective characteristics of costus sp. phytochemical contents, as well as the availability of amino acids, might explain the significant rise in serum albumin and total protein in the hexane extract costus sp. treated group [44].

Also, metformin significantly increases the levels of serum globulin, albumin, and total protein. This rise might be due to the healing of hepatic injury. The activation of the enzyme that catalyses amino acid transamination results in increased hepatic amino acid absorption, stimulation of amino acid incorporation into protein, and decreased proteolysis [45].

STZ (2-deoxy-2(3-methyl-3-nitrosoureido)-D-glucopyranose) is a nitrosourea analogue that has been used to induce diabetes in animal studies. The specific mechanism of STZ activity in pancreatic -cells was previously revealed in review papers. The powerful diabetogenic effect of STZ is caused by damage to the pancreatic B-cells, which are the body's only source of insulin. STZ is relatively selective for these cells [46]. The glucose moiety of STZ (methyl nitrosourea) permits it to enter insulin-secreting cells by the glucose transporter GLUT2, however it is the nitrosoamide moiety that causes damage. The DNA damage produced by STZ exposure in insulin-secreting cells has received a lot of attention. STZ induces DNA alkylation and fragmentation in insulinoma cells and β-cells, according to multiple in vitro studies (RINm5F) [47]. This mechanism is the main reason of the shrunken islet cell region with significant cell vacuolization, apoptosis, and intra-insular edema and few inflammatory cells. This explains the diabetic control group's increased fasting glucose level, -glucosidase inhibitory effect, and reduction in insulin level.

After 30 days of therapy, C. Speciosus hexane extract decreased the rise in blood glucose levels and -glucosidase inhibitory levels in STZ-induced diabetic rats. That is because the extract contains eremanthin and costunolide, which are illustrated by GC/MS analysis. There are a number of possible mechanisms through which eremanthin and costunolide may act like insulin in peripheral tissues, including enhancing glucose uptake metabolism, blocking hepatic gluconeogenesis, and increasing glucose absorption into muscle and adipose tissues [4, 47, 48].

According to our results, eremanthin works largely by stimulating insulin release. It dramatically increased serum insulin levels in STZ-treated rats given C. speciosus hexane extract, but had no influence on the levels of insulin in normal control rats given C. speciosus hexane extract. [49]. And Costunolide's mechanism of action might be that it increases insulin secretion by lowering nitric oxide synthase production in beta islets. Costunolide has been demonstrated to inhibit nitric oxide synthase expression, aiding in the repair of diabetic secretary deficits [4]. That is why, as compared to the diabetic group, the diabetic group treated with costus hexane extract showed modest regeneration of beta cells, larger islet cells, and intra-insular edoema with mild cell vacuolization of pancreatic cells, as well as an increase in insulin level. The costus hexane extract had no effect on normal beta cells and consequently insulin levels in the control group.

Metformin has a minor effect on glucose absorption via the gastrointestinal system, although it does slow it down significantly. Metformin's polarity necessitates the use of cellular membrane transporters absorption and secretion. The principal metformin transporters are mediated by an increase in the insulin receptor's tyrosine kinase activity and increased activity and translocation of glucose transporters to the serum membrane, such as GLUT4 (also known as SLC2A4). Metformin has also been linked to increased insulin receptor expression and a better capacity to repair enzymatic pathways involved in insulin signaling [50]. This mode of action is the primary reason for the decrease in fasting glucose and -glucosidase inhibitory levels in the diabetic group treated with metformin after 30 days.

When compared to the diabetic group, diabetic rats treated with metformin showed a significant increase in serum insulin hormone as evidenced by histopathological examination of the pancreas, which revealed slight regeneration of beta cells, enlarged islet cells, and intra-insular edoema with

mild cell vacuolization of pancreatic cells. Metformin's capacity to reestablish a normal secretory pattern in rat pancreatic islets with diminished function is responsible for this. Metformin was found to have a direct and beneficial effect on beta cell secretory function by correcting intracellular abnormalities in FFA metabolism and glucose and restoring a normal secretory pattern in rat pancreatic islets with impaired function [50]. However, it had no effect on the metformin-treated normal control group.

According to the STZ-induced diabetes group's blood lipid profile, the STZ-induced diabetic group had higher TG and TC levels, and a drop in HDL-C levels. High levels of lipids in diabetic blood are mostly the result of an increased metabolic rate of fatty acids liberated from fat depots in the periphery due to insulin's ability to block hormone-sensitive lipase. Insulin shortage or insulin resistance may be the cause of dyslipidemia because insulin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coA) reductase, a crucial rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. At initially, acute insulin deficiency increases the mobilisation of free fatty acids from adipose tissue. As a result, the creation of LDL particles high in cholesterol rises. HDL is a lipoprotein that has anti-atherogenic properties. As a cholesterol transporter, It delivers cholesterol to the liver from peripheral tissues [51].

A lack of lipoprotein lipase activity in diabetics has been linked to a large increase in triglycerides in the blood [52].

Hexane extract of costus sp. treatment resulted in significant decreases in triacylglycerol, LDL-cholesterol, and total cholesterol levels when compared to the STZ-treated group. HDL cholesterol levels increased significantly as compared to the STZ-treated group. Although the mechanism of the hypolipidemic effect of these extracts is not yet known, it may, however, be attributed to phytochemical constituents inherent in them. The phyto-constituents in costus extract may have lowered blood lipids by competing with cholesterol production in the liver, perhaps by blocking the important enzyme hydroxyl methyl glutaryl coenzyme (HMG Co-A) at the regulatory site during cholesterol biosynthesis. HDL-c plays a direct part in the atherogenic process, which is consistent with findings from therapeutic interventions aimed at increasing HDL-c levels [53].

It is probable that the increase in HDL cholesterol following intake of hexane extract is due to the rise in the activity of lecithin cholesterol acyl transferase, which may assist to manage blood lipid levels. C. speciosus crude extracts administered orally lowered blood lipids and LDL cholesterol levels in rats with type 2 diabetes [10].

Following treatment with a hexane extract of costus sp., the levels of TG and TC in diabetic rats were drastically lowered. These effects might be attributed to lower activity of cholesterol biosynthesis enzymes and/or low lipolysis levels, both of which are controlled by insulin. The hexane extract of costus sp. induces a significant fall in blood HDL levels when compared to the control level, supporting the extract's hypolipidemic effect [54].

Metformin therapy led in significant decreases in total cholesterol, LDL-cholesterol, and triacylglycerol levels upon compared to the STZ-treated group. HDL cholesterol levels increased significantly as compared to the STZ-treated group. Metformin's augmentation of the lipid profile is due to the activation of AMPK in hepatic cells; as a result, Acetyl-CoA carboxylase (ACC) activity is lowered. [55].

When comparing the diabetic group to the normal control group, the biochemical findings of the kidney function tests indicated an increase in blood urea and creatinine levels. The kidneys and livers of STZ-induced diabetic rats show pathological alterations. According to several research There has been a large increase in the rate of kidney cell destruction (nephropathy) in diabetic diseases. Hyperglycemia produces a rise in free radical generation due to glucose auto-oxidation, and this rise in free radicals might injure kidney cells [56].

In diabetic nephropathy, an uncontrolled rise in blood glucose levels in the kidney produces pathophysiological processes such as glycosylation of basement membrane collagen and reduced renal function, leading in high levels of blood urea and creatinine (also known as diabetic microvascular complication) [57].

While diabetes controls had considerably greater levels of urea and creatinine, diabetic animals treated the costus sp. hexane extract had significantly lower levels of urea and creatinine. This decrease might be attributed to increased renal function as a result of lower glucose levels and consequent glycosylation [58].

In terms of blood urea, the serum creatinine levels of diabetic rats treated with metformin were significantly lower than those of the diabetic group. Metformin treatment alone improved the majority of these renal dysfunctions, demonstrating that metformin therapy protects rats with T2DN. Metformin was beneficial in treating decreased glomeruli, tubule dilation, renal capsule and GBM thickening [59].

TNF-α is generated mostly by macrophages. TNF-α inhibits kinase activity in the insulin-signaling pathway, which influences insulin signalling intracellularly in adipose, endothelial cells, skeletal muscle, and other insulin-responsive tissues [60]. Studies have shown that TNF-α may cause insulin resistance. TG and VLDL concentrations rise and lipolysis occurs in rat, mouse, and human fat cells when TNF-α is present [61, 62]. Insulin receptor tyrosine kinase activity is inhibited at low concentrations by TNF-α; at higher concentrations, TNF-α can also inhibit insulin receptor Glut-4 and IRS-1 expression, as well as increase the phosphorylation of serine 307 in IRS-1, impairing its ability to bind to the insulin receptor and initiate downstream signalling [60]. Obesity, poor glucose tolerance, and type 1 and 2 diabetes all increase circulating IL-6 levels [62]. Through their different actions, IL-6, TNF-α, and CRP contribute significantly to vascular inflammation and insulin resistance [63].

IL-6, TNF-α, and CRP blood concentrations were considerably higher in diabetic rats. TNF-α, and IL-6 production is increased in response to oxidative stress. TNF-α not only enhances adipocyte lipolysis, but it also has a negative impact on the insulin signalling pathway by altering the tyrosine/serine phosphorylation of insulin receptor substrate. CRP is a major inflammatory factor that is influenced by IL-1, IL-6, and TNF-α and is generated by the liver in response to inflammation. [61].

These phenolic acids' existence in C. pictus leaf extract might possibly be responsible for the extract's beneficial effects. Flavonoids, particularly quercetin, have been extensively researched and shown to lower Low-grade inflammation and oxidative stress. Researchers have shown that quercetin reduces the production of the pro-inflammatory cytokine TNF-α in peripheral blood mononuclear cells. This is caused by changes in the ERK and NF-B cascades [62].

TNF-α alters the insulin response in insulin-responsive tissues, causing systemic insulin resistance. It induces serine phosphorylation of IRS-1, which blocks the subsequent signalling cascade by suppressing insulin-mediated tyrosine phosphorylation. Flavonoids have also been reported to decrease IL-6 production through regulating p38 and PKC phosphorylation. TNF-α and CRP levels in the blood were considerably lower in rats given C. pictus extract. [63].

The anti-inflammatory effects of metformin are hypothesised to be mediated by AMP-activated protein kinase activation, antioxidant activity, and insulin sensitivity. Metformin, an AMPK activator, has been demonstrated to lower systemic inflammation in people with moderate metabolic syndrome. In diabetic rats, activation of AMPK as a result of a considerable rise in MNCV and a reduction in TNF-α, IL-6, and CRP as examples of inflammatory indicators. [64]. When compared to untreated diabetic rats, our data showed that metformin therapy lowered inflammatory responses, with the lower levels of IL-6, CRP, and TNF-α.

There is a change in glucose, lipid, and protein metabolism in diabetes mellitus, which raises the risk of major vascular issues. Oxidative stress is generated by an increase in mitochondrial reactive oxygen species (ROS) production, glucose autoxidation, and non-enzymatic protein glycation [65]. Due to enhanced ß-oxidation, and mitochondrial uncoupling elevated free fatty acids (FFAs) can induce oxidative stress, resulting in an increase in the production of reactive oxygen species (ROS). Increased reactive oxygen species (ROS) production (or inadequate ROS clearance) contributes to the pathophysiology of diabetes problems [66]. Advanced glycation end products (AGEs) and re-

ceptors for AGE (RAGE), protein kinase C (PKC), polyol. B (NF. B), pathway, nuclear factor NH2-terminal Jun kinases/stress activated protein kinases (JNK/SAPK), p38 mitogen-activated protein (MAP) kinase, and hexosamine pathway are all examples of cellular stress-sensitive pathways. It has been established that the activation of these pathways is linked to the late-stage problems of diabetes, insulin resistance, and malfunction in -cells [67].

In diabetic patients, hypoinsulinemia leads to an increase in the activity of the enzyme fatty acyl coenzyme A oxidase, which in turn encourages the ß-oxidation of fatty acids and lipid peroxidation. The fluidity of membranes is reduced, and the activity of enzymes and receptors that are linked to membranes is altered when there is an increase in the amount of lipid peroxidation that occurs. Lipid peroxidation products are toxic to most cells in the body and have been linked to a number of illnesses, including atherosclerosis and brain damage. MDA levels in the serum of diabetic individuals were found to be considerably higher in our study [68].

Because it is concerned with immediate removal of reactive oxygen species, superoxide dismutase is considered a main enzyme. SOD is an essential defensive enzyme that catalyses superoxide radical dismutation. CAT is a ubiquitous antioxidant enzyme present in practically all oxygen-using biological tissues. The enzyme, which employs either manganese or iron as a catalyses, cofactor, the reduction or breakdown of hydrogen peroxide (H_2O_2) to water and molecular oxygen, thereby finishing the detoxifying process that SOD imitated [69]. In the reduction of hydrogen peroxide, GPx was thought to be physiologically important. A decrease in GPx activity in diabetic rats might potentially be attributed to radical-induced inactivation and enzymatic glycation. [58]. In diabetic rats, CAT and SOD activity levels in the kidney and liver tissues were reduced, which may result in a number of negative consequences induced by the buildup of superoxide radicals ($O_2\cdot-$) and hydrogen peroxide (H_2O_2) [70].

The administration of hexane extract of costus sp. increases the number of insulin levels and beta cells in blood, and insulin was shown to boost cell ability to metabolise glucose while also controlling betaoxidation of hydrogen peroxide and fatty acids generation. The oral administration of a costus sp. hexane extract increased antioxidant enzyme levels while decreasing MDA levels in STZ-injected rats. [70].

The presence of phytoconstituents in C. speciosus rhizome, such as flavonoids and phenolic compounds, may explain its antioxidant action. [71]

The presence of essential oils, which have antioxidant activity, the normal level of glucose, insulin, lipid profile, and beta cell regeneration in the pancreas due to the administration of hexane extract of costus sp. As a result, the levels of MDA, SOD, CAT, and GP_X enzymes returned to normal levels, as shown in our present study.

Metformin significantly normalized most of the altered metabolic and oxidative stress parameters. Thus, it is probable that metformin's antihyperglycemic and anti-oxidant actions in STZ-diabetic rats are attributable to Met-insulin-like activity [72]. As a result, the levels of MDA, SOD, CAT, and GP_X enzymes returned to normal levels, as shown in our present study.

5. Conclusion

Diabetic rats had great disturbances in liver functions, kidney functions, immunological parameters, antioxidant parameters, and histopathological examination of the pancreas. Metformin and hexane extract of costus sp. treated groups revealed enhancement and improvement of disturbances of liver functions, kidney functions, immunological parameters, antioxidant parameters, and histopathology of the pancreas. From the obtained results, it could be concluded that hexane extract of costus sp. and metformin exert therapeutic effects on diabetes and diminish the side effects and complications of diabetes. Both hexane extract of costus sp. and metformin have the same effect on diabetes as well as the side effects and complications of diabetes. Metformin and hexane extract of costus sp. have no side effect on diabetes or normal rats.

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