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Assessment of the Biochemical Efficacy of Gold *Saussurea costus* Nano-extract against Diabetes-Induced Neurochemical Alterations in the Brain of Rats

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ABSTRACT

The objective of this research was to demonstrate the biological effectiveness of gold nanoparticles (Au-NPs) biosynthesized using *Saussurea costus* extract in mitigating adverse effects in the brain of streptozotocin (STZ)-induced diabetic rats. The levels of glucose and insulin hormones were measured, along with hematological indices, as well as conventional biochemical assays for liver, heart, and kidney functions. The brain tissue homogenates were analyzed for lipid peroxidation products (LPO) and total protein carbonyl content (TPC), as well as antioxidant system indicators including reduced glutathione (GSH), total antioxidant capacity (TAC), and the activities of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Additionally, measurements were taken for β-amyloid (Aβ) content, acetylcholinesterase (AChE) enzyme activity, and inflammatory markers interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). The study found that diabetic rats treated with native *S. costus* extract and gold nano-extract showed a significant ($p \leq 0.05$) increase in hematological measurements but a decrease in conventional biochemical assays. Insulin hormone levels decreased, leading to a significant reduction in glucose levels with both extracts. Furthermore, both the native extract and gold nano-extract significantly increased enzymatic and non-enzymatic antioxidants while decreasing peroxidation reaction products (LPO and TPC) and inflammatory markers (TNF-α and IL-1β). In conclusion, both native *S. costus* extract and gold nano-extract showed improvements in elevated measurements caused by STZ-induced diabetes. However, only the gold nano-extract was able to bring all measurements back to normal levels.

Keywords: Diabetes mellitus, *Saussurea costus*, Oxidative stress, Inflammation, Green Nanotechnology

1. Introduction

Hyperglycemia followed by insulin resistance is a marker of diabetes mellitus (DM), a chronic metabolic disease that is regarded as a leading cause of mortality worldwide (Borai *et al*., 2016; Młynarska *et al*., 2025). The International Diabetes Federation (IDF) conducted an investigation in 2017, which predicted that the rate of disease incidence will rise steadily over the coming years (Hussien *et al*., 2024a).

Due to changes in glycometabolism, DM causes a number of issues in both the central nervous system and peripheral organs (Tartau *et al*., 2025). Diabetes may not have any noticeable effects on the brain, but over time, a steady reduction in the blood flow to neurons can lead to brain shrinkage (Zhang *et al*., 2022). Diabetes increases the risk of neurological problems, including Alzheimer's disease, diabetic neuropathy, cognitive impairment, and other neurodegenerative disorders (Biessels and Despa, 2018).

DM causes changes in the structure, function, and morphology of the brain. According to reports, ischemic brain injury in DM may be mediated via the mitochondria-dependent cell death pathway and mitochondrial malfunction. As a result, brain tissue was chosen for this investigation (Liao et al., 2025a). Numerous neuropsychiatric comorbidities, including bipolar disorder, schizophrenia, and

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depression, are frequently associated with DM (Chen *et al*., 2022). One problem linked to disability and the onset of dementia, depression, and Alzheimer's disease is thought to be hippocampal dysfunction, including memory loss (Roux *et al*., 2021). Diabetic encephalopathy, diabetes-associated cognitive decline, cerebral impairment, and central neuropathy are some of the names for mild to severe diabetes-related cognitive dysfunction (Karunathilaka *et al*., 2024).

No single medication is completely successful in treating the condition due to its severe micro- and macrovascular consequences. Consequently, further research is required to find novel drugs that can slow the progression of the disease and minimize potential side effects (Guan *et al*., 2024). It was suggested that anti-diabetic medications made from plants are usually considered to be less expensive and less hazardous than their synthetic counterparts (Ramadaini *et al*., 2024).

Many diseases, including tenesmus, dyspepsia, diarrhea, vomiting, inflammation, asthma, bronchitis, cough, throat infections, inflammatory diseases, ulcers, and stomach problems, can be treated with *Saussurea costus*, also called Saussurea lappa. It has antiviral properties as well (Ali *et al*., 2021; Elnour and Abdurahman, 2024). Terpenes, anthraquinones, alkaloids, flavonoids, dehydrocostus lactone, and costunolide are among the many phytoconstituents found in *S. costus* that have a variety of biological characteristics. Its antidiabetic, antihypertensive, antihepatotoxic, immunostimulant, anthelmintic, anti-inflammatory, antitumor, antiulcer, antimicrobial, and antifungal properties are all attributed to these ingredients (Kumari *et al*., 2024).

The methanolic extract of *S. costus* root exhibited strong antioxidant activity due to its high phenol and flavonoid content. These secondary metabolites have beneficial biological effects as they contain a variety of functional groups (Binobead *et al*., 2024). Deabes *et al*. (2021) conducted a study to evaluate the efficacy of this plant extract in lowering the hepato- and neurotoxicity produced by chlorpyrifos in rats.

Large molecular weights characterize the majority of physiologically active substances. Their slow absorption limits their effectiveness and bioavailability as they are unable to pass through the cellular membrane efficiently (Liu *et al*., 2024). Combining natural compounds with nanotechnology can improve their effectiveness by reducing toxicity and boosting bioavailability. This strategy aims to reduce toxicity and the need for frequent dosing (Zhuo *et al*., 2024). Metal nanoparticles (M-NPs), which are considered the most promising method for enhancing the intrinsic stability of plant extracts, were incorporated into the extract to create nano-extracts (Adeyemi *et al*., 2022). When compared to plant extract alone, the biosynthesized M-NPs using natural extracts increased biological activity at lower doses, enhancing their usefulness (Antunes Filho *et al*., 2023). From this perspective, the goal of this study was to evaluate the biological effectiveness of gold nanoparticles (Au-NPs) produced with methanolic extract from *S. costus* in mitigating the harmful effects on the brains of diabetic rats.

2. Materials and Methods

2.1. Administration of the extract

The median lethal doses (LD_{50}) of native *S. costus* extract and gold nano-extract were determined by Hussien *et al*. (2024b) to be around 7333.33 and 11333.33 mg/kg, respectively. Therefore, in the *in vivo* investigation, therapeutic doses of 366.67 and 566.67 mg/kg b.w., respectively, were selected for oral administration, which were determined to be 1/20 of LD_{50}.

2.2. Induction of diabetes mellitus

An intraperitoneal (*i.p.*) injection of 60 mg/kg body weight of streptozotocin (STZ) solution, freshly prepared in citrate buffer (100 mM, pH 4.5), was administered to rats that had fasted for the entire night (Archana *et al*., 2001). Massive glycosuria and hyperglycemia appeared a few days after the STZ solution was injected. The fasting blood glucose level was measured 72 hours following the STZ injection to confirm the induction procedure. A rat was deemed to have diabetes if its blood glucose level was more than 200 mg/dl.

2.3. Experimental Design

Fifty rats were divided into five groups of ten each. **The control group** was given distilled water and a normal diet for 21 days. The study involved different treatment groups. **The *S. costus* extract group** received the extract orally at a dose of 366.67 mg/kg b.w. for 21 days. **The Gold *S. costus* nano-**

extract group received the nano-extract orally at a dose of 566.67 mg/kg b.w. for 21 days. **The diabetic group** received a single dose of STZ solution *i.p.* and were sacrificed after 21 days. The diabetic rats treated with *S. costus* extract or gold *S. costus* nano-extract were injected with STZ solution and treated with the respective extracts for 21 days.

2.4. Collection of blood samples and preparation of tissues

After the last dose of treatment, the animals underwent an 18-hour fast and anesthesia. Blood samples were collected from the retro-orbital plexus in heparinized tubes for hematological analysis. The remaining blood samples were centrifuged at 3000 rpm for 15 minutes after clotting, and the separated sera were stored at -20 °C for biochemical analysis. The brain tissues were extracted and cleaned in ice-cold saline following cervical dislocation. Autopsied fragments were homogenized in potassium phosphate buffer (pH 7.4) and centrifuged at 3000 rpm for 10 minutes. The transparent supernatants were stored at -80 °C for biochemical investigations.

2.5. Hematological and biochemical assays

Heparinized blood samples were analyzed using an automated blood analyzer to measure various hematological parameters such as red blood cell (RBC) and white blood cell (WBC) counts. Lipid profiles, including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C), were assessed using commercially available kits. Conventional biochemical assays were conducted to evaluate liver, heart, and kidney functions, as well as glucose levels in serum samples. The calculation of low-density lipoprotein-cholesterol (LDL-C) followed the method outlined by Schumann and Klauke (2003). Plasma insulin levels were determined using a sandwich enzyme-linked immunosorbent assay kit (ELISA) from Boehringer Mannheim, Mannheim, Germany.

2.6. Biochemical assays in supernatants of tissues homogenates

Methods for quantifying oxidative stress markers in brain tissue homogenates, such as reduced glutathione (GSH) and total antioxidant capacity (TAC), were developed by Beutler *et al*. (1963) and Koracevic *et al*. (2001), respectively. The antioxidant enzymes glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were measured in units per gram of tissue using the methods described by Paglia and Valentine (1967), Aebi (1984), and Sun *et al*. (1988), respectively. Furthermore, the levels of total protein carbonyl content (TPC) and lipid peroxidation product (LPO) were measured in nmol/mg and nmol/g tissue, respectively, using the methods described by Levine *et al*. (1994) and Ohkawa *et al*. (1979), respectively. In addition to the β-amyloid (Aβ) content, the inflammatory markers tumor necrosis factor-α (TNF-α) (Engelmann *et al*., 1990) and interleukin-1β (IL-1β) (March *et al*., 1985) were measured as pg/g using the quantitative sandwich enzyme immunoassay (ELISA) approach. The activity of the acetylcholinesterase (AChE) enzyme, which was expressed as nmol ACh hydrolyzed/min/mg protein, was measured using the Ellman Method, proposed by Ellman *et al*. (1961) and modified by Gorun *et al*. (1978).

2.7. Statistical Analysis

The analyses in the tables and figures show as mean ± standard error (SE). One-way ANOVA and Bonferroni test were used for post-hoc comparisons. A "p" value < 0.05 indicates a significant difference.

3. Results

3.1. Hematological and biochemical measurements

According to the data in Table 1, administering the native *S. costus* extract and the gold nano-extract did not significantly alter any of the hematological measurements, including WBC levels, differential counts (Lymph., Mono., Gran.), and indices of red blood cell indices (RBCs, HB, HCT, and PLT). The hematological parameters were significantly ($p \leq 0.05$) lower in the STZ-induced diabetic group compared to the control group. Treatment of diabetic rats with the native *S. costus* extract led to an improvement in their hematological parameters compared to diabetic rats, but levels did not return to normal. However, administration of the gold nano-extract resulted in the improvement and normalization of hematological measures.

Table 1: Effect of gold *S. costus* nano-extract on the changes in various hematological parameters caused by streptozotocin (STZ) in diabetic rats.

		C.	*S. costus* Ext.	Gold Nano-Ext.	Diabetic	Diabetic group treated with	
						S. costus Ext.	Gold Nano-Ext.
Formed elements	**RBCs (10^6/ul)**	7.31 ± 0.06	7.32 ± 0.06	7.30 ± 0.06	4.87 ± 0.04[a]	5.89 ± 0.04[ab]	7.27 ± 0.05[b]
	HB (g/dl)	16.24 ± 0.06	16.27 ± 0.06	16.22 ± 0.06	10.83 ± 0.04[a]	13.10 ± 0.05[ab]	16.16 ± 0.06[b]
	HCT (%)	45.07 ± 0.05	45.16 ± 0.05	45.02 ± 0.05	30.05 ± 0.03[a]	36.36 ± 0.04[ab]	44.85 ± 0.05[b]
	PLT (10^3/ul)	489.27 ± 1.60	490.24 ± 1.60	488.68 ± 1.60	326.18 ± 1.07[a]	394.68 ± 1.29[ab]	486.84 ± 1.59[b]
	WBCs (10^3/ul)	11.05 ± 0.02	11.07 ± 0.02	11.04 ± 0.02	7.37 ± 0.01[a]	8.91 ± 0.02[ab]	11.00 ± 0.02[b]
Differential count	**Lymp. (10^3/ul)**	8.91 ± 0.01	8.93 ± 0.01	8.90 ± 0.01	5.94 ± 0.01[a]	7.19 ± 0.01[ab]	8.87 ± 0.01[b]
	Mono. (10^3/ul)	0.80 ± 0.01	0.80 ± 0.01	0.80 ± 0.01	0.54 ± 0.01[a]	0.65 ± 0.01[ab]	0.80 ± 0.01[b]
	Gran. (10^3/ul)	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	0.34 ± 0.01[a]	0.41 ± 0.01[ab]	0.50 ± 0.01[b]

Data were derived from five replicates and presented as mean ± SE, a: significantly different from the control group, b: significantly different from the diabetic group at $p \leq 0.05$.

Based on the data presented in Fig. 1, the STZ injection led to a significant ($p \leq 0.05$) increase in blood glucose levels and a decrease in insulin levels compared to the control group. Both the gold nano-extract and the native *S. costus* extract significantly ($p \leq 0.05$) increased insulin levels and reduced glucose levels compared to the STZ-induced diabetic group. However, the gold nano-extract was able to restore the levels to normal.

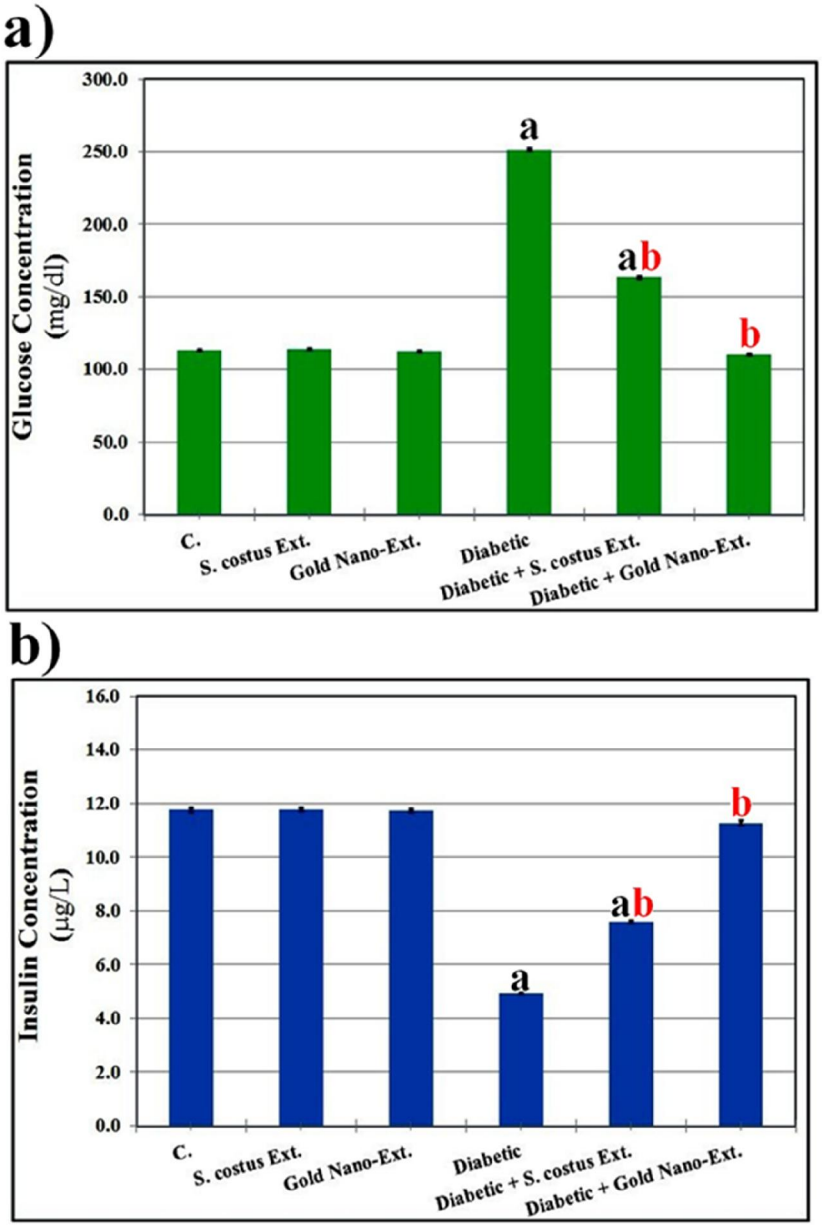


Fig. 1: Effect of gold *S. costus* nano-extract on the changes in **a)** glucose level and **b)** insulin level in sera caused by streptozotocin (STZ) in diabetic rats. Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.

There were no significant differences in any of the biochemical parameters between the native *S. costus* extract and the gold nano-extract compared to the control group (Table 2). The injection of STZ resulted in a significant ($p \leq 0.05$) decrease in HDL-C and a significant ($p \leq 0.05$) increase in the activities of liver enzymes (ALT, AST, ALP, and GGT) and cardiac enzymes (CK and LDH), as well as lipid profile measures (total cholesterol, TGs, and LDL-C). The diabetic rats induced by STZ showed a significant ($p \leq 0.05$) decrease in protein content (total protein and albumin) and a significant ($p \leq 0.05$)

Table 2: Effect of gold *S. costus* nano-extract on the changes in various biochemical parameters caused by streptozotocin (STZ) in diabetic rats.

		C.	*S. costus* Ext.	Gold Nano-Ext.	Diabetic	Diabetic group treated with	
						S. costus Ext.	Gold Nano-Ext.
Liver	**ALT (**U/L**)**	49.63 ± 0.01	49.73 ± 0.01	49.57 ± 0.01	103.40 ± 0.03^{a}	67.21 ± 0.02^{ab}	48.32 ± 0.01^{b}
	AST (U/L**)**	71.84 ± 0.01	71.99 ± 0.01	71.76 ± 0.01	149.67 ± 0.02^{a}	97.29 ± 0.01^{ab}	69.94 ± 0.01^{b}
	ALP (U/L**)**	110.45 ± 0.01	110.67 ± 0.01	110.32 ± 0.01	230.11 ± 0.02^{a}	149.57 ± 0.02^{ab}	107.53 ± 0.01^{b}
	GGT (U/L**)**	28.06 ± 0.01	28.11 ± 0.01	28.02 ± 0.01	58.45 ± 0.02^{a}	37.99 ± 0.01^{ab}	27.31 ± 0.01^{b}
Kidney	**Urea (**mg/dl**)**	43.85 ± 0.02	43.94 ± 0.02	43.80 ± 0.02	91.35 ± 0.05^{a}	59.38 ± 0.03^{ab}	42.69 ± 0.02^{b}
	Creat. (mg/dl**)**	1.72 ± 0.01	1.72 ± 0.01	1.71 ± 0.01	3.57 ± 0.02^{a}	2.32 ± 0.01^{ab}	1.67 ± 0.01^{b}
	T. Protein (g/dl**)**	9.69 ± 0.01	9.71 ± 0.01	9.68 ± 0.01	4.65 ± 0.01^{a}	7.16 ± 0.01^{ab}	9.77 ± 0.01^{b}
	Albumin (g/dl**)**	4.77 ± 0.01	4.78 ± 0.01	4.76 ± 0.01	2.29 ± 0.01^{a}	3.52 ± 0.01^{ab}	4.81 ± 0.01^{b}
Lipid	**TC (**mg/dl**)**	102.04 ± 0.02	102.24 ± 0.02	101.92 ± 0.02	212.59 ± 0.05^{a}	138.18 ± 0.03^{ab}	99.34 ± 0.02^{b}
	T.Gs (mg/dl**)**	102.05 ± 0.01	102.25 ± 0.01	101.93 ± 0.01	212.61 ± 0.03^{a}	138.20 ± 0.02^{ab}	99.35 ± 0.01^{b}
	HDL-c (mg/dl**)**	21.53 ± 0.02	21.58 ± 0.02	21.51 ± 0.02	6.72 ± 0.01^{a}	10.34 ± 0.01^{ab}	22.12 ± 0.02^{b}
	LDL-c (mg/dl**)**	60.10 ± 0.03	60.22 ± 0.03	60.02 ± 0.03	125.20 ± 0.05^{a}	41.52 ± 0.06^{ab}	58.51 ± 0.03^{b}
Heart	**CK (**U/L**)**	80.24 ± 0.03	80.39 ± 0.03	80.14 ± 0.03	167.16 ± 0.06^{a}	108.65 ± 0.04^{ab}	78.11 ± 0.03^{b}
	LDH (U/L**)**	255.35 ± 0.09	255.85 ± 0.09	255.03 ± 0.09	531.97 ± 0.20^{a}	345.78 ± 0.13^{ab}	248.58 ± 0.09^{b}

Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.

increase in urea and creatinine levels, indicating renal dysfunction. Treatment with *S. costus* extract improved all biochemical measurements but did not fully restore them to normal levels. In contrast, the gold nano-extract completely normalized the biochemical measurements.

3.2. Markers of oxidative stress

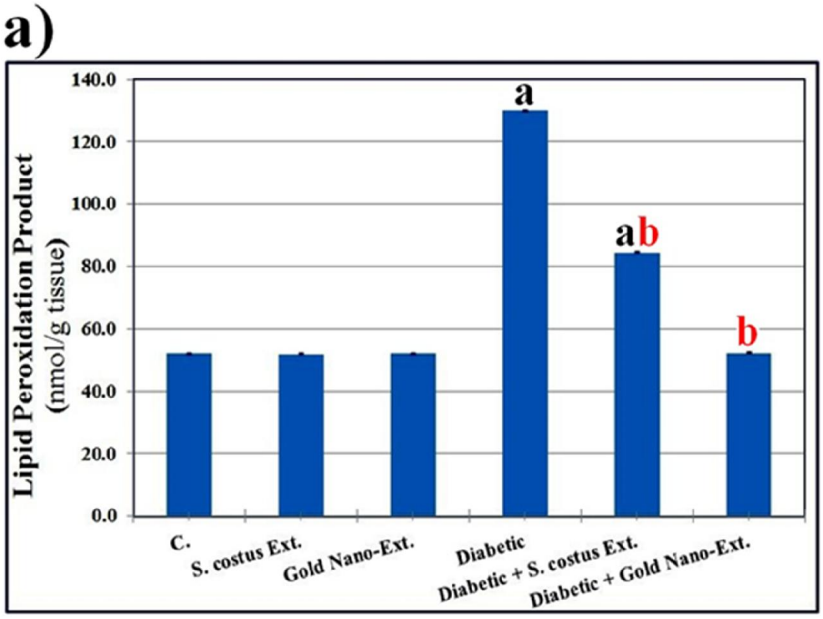
Based on the findings from Table 3, the brain tissue homogenates of diabetic rats induced by STZ exhibited a significant ($p \leq 0.05$) decrease in TAC and GSH levels, along with reduced activity of antioxidant enzymes (SOD, CAT, and GPx). Treatment with *S. costus* extract and gold nano-extract resulted in a significant ($p \leq 0.05$) improvement in antioxidant parameters compared to the diabetic group. Particularly, the gold nano-extract fully normalized the levels of these markers.

The STZ injection resulted in significantly higher levels of LPO and TPC compared to the control group in terms of peroxidation products (Fig. 2), with a *p*-value of ≤0.05. In comparison to the STZ-induced diabetic group, the diabetic rats treated with *S. costus* extract showed a significant reduction ($p \leq 0.05$) in the levels of these toxic substances, although they did not fully return to normal levels. However, treatment with the gold nano-extract significantly decreased the levels of LPO and TPC, bringing them back to normal values.

Table 3: Effect of gold *S. costus* nano-extract on the changes in the levels of the enzymatic and non-enzymatic antioxidants in the brain tissue caused by streptozotocin (STZ) in diabetic rats.

	C.	*S. costus* Ext.	Gold Nano-Ext.	Diabetic	Diabetic group treated with	
					S. costus Ext.	Gold Nano-Ext.
TAC (µmol/g**)**	9.80 ± 0.01	9.82 ± 0.01	9.82 ± 0.02	3.90 ± 0.02^{a}	6.03 ± 0.01^{ab}	9.75 ± 0.01^{b}
GSH (mg/g tissue**)**	165.00 ± 0.07	165.32 ± 0.07	164.79 ± 0.07	66.00 ± 0.03^{a}	101.54 ± 0.04^{ab}	164.18 ± 0.07^{b}
SOD (IU/g tissue**)**	51.98 ± 0.01	52.08 ± 0.01	51.92 ± 0.01	20.79 ± 0.01^{a}	31.99 ± 0.01^{ab}	51.72 ± 0.01^{b}
CAT (IU/g tissue**)**	89.23 ± 0.02	89.40 ± 0.02	89.12 ± 0.02	35.69 ± 0.01^{a}	54.91 ± 0.01^{ab}	88.78 ± 0.02^{b}
GPx (IU/g tissue**)**	69.83 ± 0.02	69.97 ± 0.02	69.75 ± 0.02	27.93 ± 0.01^{a}	42.97 ± 0.01^{ab}	69.48 ± 0.02^{b}

Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.



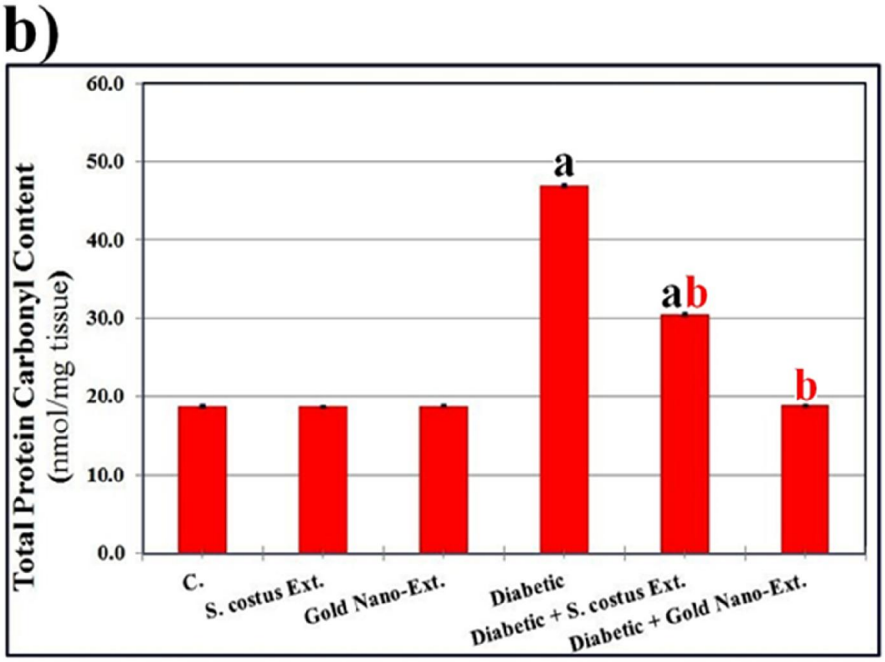


Fig. 2: Effect of gold *S. costus* nano-extract on the changes in **a)** Lipid peroxidation products and **b)** Total protein carbonyl content in the brain tissue caused by streptozotocin (STZ) in diabetic rats. Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.

3.3. Markers of inflammatory reactions

Compared to the control group, diabetic rats exhibited significantly ($p \leq 0.05$) higher levels of inflammatory markers (TNF-α and IL-1β) in their brains (Table 4). The diabetic rats treated with *S. costus* extract showed significantly lower levels of these markers compared to the STZ-induced diabetic group, although they did not completely return to normal levels. However, treatment with the gold nano-extract significantly ($p \leq 0.05$) reduced the levels of these markers and restored them to normal levels. The activity of the AChE enzyme and β-amyloid (Aβ) is closely associated with the integrity of brain tissue. Compared to the control group, the tissue of STZ-induced diabetic rats showed significantly ($p \leq 0.05$) higher levels of these measurements (Fig. 3). Treatment of diabetic rats with native *S. costus* extract did not lead to any noticeable improvement in these parameters. However, administration of gold nano-extracts improved brain integrity by reducing these parameters and restoring them to normal levels.

Table 4: Effect of gold *S. costus* nano-extract on the changes in the levels of the markers of the inflammatory reactions in the brain tissue caused by streptozotocin (STZ) in diabetic rats.

	C.	*S. costus* Ext.	Gold Nano-Ext.	Diabetic	Diabetic group treated with	
					S. costus Ext.	Gold Nano-Ext.
TNF-α (pg/g tissue)	262.90 ± 0.39	263.42 ± 0.39	262.58 ± 0.39	302.33 ± 0.45^{a}	287.94 ± 0.43^{ab}	265.20 ± 0.39^{b}
IL-1β (pg/g tissue)	137.06 ± 0.24	137.33 ± 0.24	136.90 ± 0.24	171.33 ± 0.30^{a}	163.17 ± 0.29^{ab}	140.43 ± 0.25^{b}

Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.

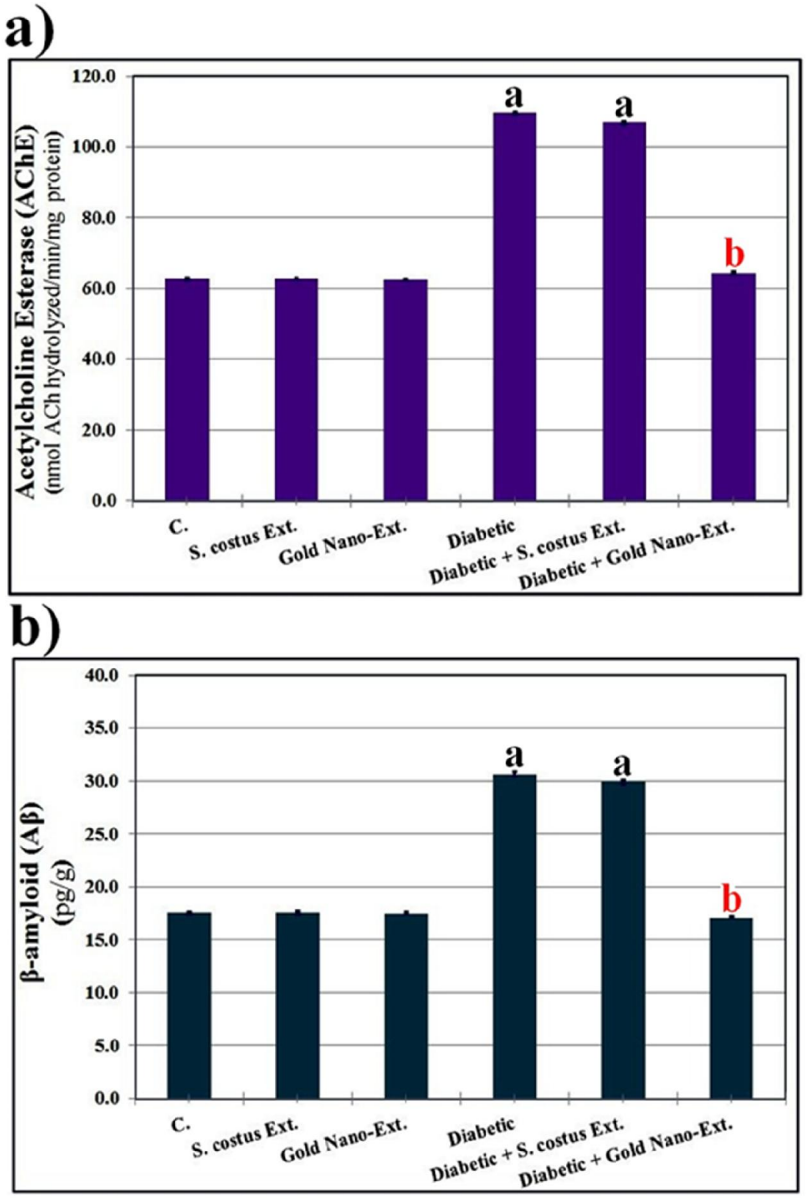


Fig. 3: Effect of gold *S. costus* nano-extract on the changes in **a)** the activity of the acetylcholine esterase (AChE) enzyme and **b)** β-amyloid (Aβ) contents in the brain tissue caused by streptozotocin (STZ) in diabetic rats. Data were derived from five replicates and presented as mean ± SE, **a**: significantly different from the control group, **b**: significantly different from the diabetic group at $p \leq 0.05$.

4. Discussion

The antibiotic streptozotocin (STZ) has a wide range of effects, including causing diabetes by specifically targeting and destroying the insulin-producing pancreatic endocrine cells and the β-cells of the islets of Langerhans. This leads to an increase in blood glucose levels due to insufficient insulin release (Ghasemi and Jeddi, 2023). Due to the negative side effects of synthetic anti-diabetic drugs, researchers have been exploring natural substances that are readily available and have hypoglycemic effects through an insulinogenic mechanism, which stimulates the β cells to release insulin (Blahova *et al*., 2021).

The RBC indices were shown to have significantly dropped in the STZ-induced diabetic rats throughout the current investigation. This may be related to the diabetogenic changes, which were linked to an increase in RBC non-enzymatic glycosylation due to lipid peroxidation. Furthermore, the diabetic rats' markedly reduced WBC count and differential blood cell count may be related to their

bone marrow's susceptibility to leukocytosis, which might explain why their immune systems aren't as strong against infection (Rehman *et al*., 2023).

STZ resulted in hyperglycemia. The demise of the pancreatic β cells, which reduces insulin levels, may be the cause of this. Consequently, insulin insufficiency causes an increase in blood glucose levels (Rad *et al*., 2022). The native *S. costus* extract exhibited the hypoglycemic effect due to its ability to inhibit the activity of carbohydrate metabolizing enzymes (α-amylase and α-glucosidase) as demonstrated by Hussien *et al*. (2024). Furthermore, the presence of active phytoconstituents, which have antioxidant properties and preserve the integrity of pancreatic cell tissues, shielding them from the assault of reactive oxygen and nitrogen species, may be linked to its hypoglycemic impact. This results in increased insulin production linked to decreased intestinal glucose absorption (Arabshomali *et al*., 2023).

The production of free radicals linked to hyperglycemia can deplete antioxidant defenses, causing oxidative damage to membranes and increased vulnerability to peroxidation products (González *et al*., 2023). Damage to the liver and kidneys was the primary disadvantage of STZ (Mohd Nasir *et al*., 2024). The liver enzymes in the current study became more active as a result of STZ. This might be a reference to changes in membrane permeability brought on by cholestatic or active liver damage. These enzymes leak out when the transport of metabolites is disrupted by changes in membrane permeability (Alqahtani *et al*., 2023). The injection of STZ led to a notable rise in CPK and LDH activities due to the infarcted myocardium caused by STZ metabolism (Malfitano *et al*., 2015).

Insulin secretion is restored when the liver and heart enzyme activity returns to normal following treatment with native *S. costus* extract. As a result, inhibition of peroxidation reactions is linked to this (Hajam *et al*., 2022). Due to the presence of Au-NPs, which increase phenolic component concentrations by regenerating polyphenolic compounds—possibly due to catalytic reaction conditions or interference from the tiny biosynthesized Au-NPs—the gold nano-extract showed more amelioration. An overestimation of the organic components in the extract might be the cause of the subsequent rise in the overall polyphenolic content (Alegria *et al*., 2018). Aboulthana *et al*. (2022) showed that using the extract for the biosynthesis of Au-NPs increased the biological effectiveness of the active phyto-constituents. This led to the gold nano-extract restoring the compromised measurements to normal levels and protecting cell membranes from reactive species. Consequently, the levels of cardiac, renal, and hepatic markers reverted to normal after treatment.Levels of total protein and albumin decreased in diabetes induced by STZ. Kilany *et al*. (2025) supported these findings by suggesting that the reduction in these levels might be due to the enhanced conversion of glycogenic amino acids into water and carbon dioxide. In STZ-induced diabetes, the natural extract of *S. costus* prevented negative impacts on kidney function. This could be attributed to the presence of fatty acids and polysaccharides, which may assist in regulating nitrogen molecules in endocrine metabolism (Cao *et al*., 2021).

In terms of lipid profile measurements, diabetes induced by STZ resulted in dyslipidemia, characterized by elevated levels of total cholesterol, triglycerides, and LDL-C, along with a decrease in HDL-C. This could be attributed to various biochemical processes, such as the activation of hormone-sensitive lipase to facilitate the release of fatty acids from triglycerides in adipocytes, and/or the activation of lipoprotein lipase to aid in the hydrolysis of triglycerides in endothelial cells (Amaechi *et al*., 2015). The presence of polysaccharides in the native *S. costus* extract has been shown to inhibit intestinal absorption, leading to reductions in cholesterol, triglycerides, and LDL-C levels (Niewold *et al*., 2012).

The compromised biochemical measurements normalized after the administration of *S. costus* extract. The presence of phenolics, chlorophylls, and carotenoids in the extract, known for their antioxidant properties, may have protected the liver, kidney, and heart tissues from reactive oxygen and nitrogen species, leading to the improvement (Ben Abdelmalek *et al*., 2025). The biosynthesized Au-NPs, with their surface reaction and high surface area-to-volume ratio, could have enhanced the interaction and scavenging activity of free radicals, resulting in increased antioxidant activity and the normalization of biochemical measurements post-treatment with the gold nano-extract (Mikhailova, 2021).

Uncertainty surrounds the pathophysiology of brain impairment caused by the prevalence of diabetes. The brain is the organ most vulnerable to inflammation and changes in blood sugar levels. Both metabolic and vascular pathways are affected by hyperglycemia, leading to disruptions in various brain regions and impaired brain function (Zhang *et al*., 2023). Cognitive impairment is a possible

consequence of diabetes in its early stages. Finding important indicators of early neural dysfunction is therefore essential (Liao *et al*., 2025b).

Consistent with the findings of Mihailović *et al*. (2021), which showed that decreased antioxidants in the brain were a result of reactive species formation from glucose autoxidation and/or non-enzymatic protein glycation, our study revealed reduced levels of TAC and GSH in the brains of diabetic rats. The increased production of reactive oxygen species (ROS) in diabetic rats led to elevated levels of LPO and TPC, indicating interactions with lipids and proteins (An *et al*., 2023). The native *S. costus* extract contains active phytoconstituents that can act as hydrogen atom donors and singlet oxygen scavengers. This leads to an increase in antioxidants, a decrease in peroxidation reaction products, and prevention of oxidative stress-induced changes while maintaining a nearly normal antioxidant status. These qualities are due to its antioxidant properties (Jomova *et al*., 2023). Additionally, the presence of Au-NPs in the nano-extract enhanced its antioxidant properties and demonstrated increased protection against ROS (David *et al*., 2024).

Most neurodegenerative disorders involve oxidative stress and neuroinflammation as their pathological markers (Simpson and Oliver, 2020). Injury to the central nervous system initiates the activation of astrocytes and microglia, resulting in the release of proinflammatory cytokines and, ultimately, neuronal death (Zhang *et al*., 2023).

Due to their unfavorable link with cognitive impairments, the current investigation found that the brains of diabetic rats had considerably higher levels of pro-inflammatory cytokines (TNF-α and IL-1β). Furthermore, the acceleration of the neurodegenerative process is tightly linked to the production of these cytokines (Piatkowska-Chmiel *et al*., 2021). The abnormal differentiation of vascular endothelial cells and perivascular macrophages led to an increase in pro-inflammatory cytokines, suggesting an exaggerated inflammatory response characterized by elevated cytokine production (Denes *et al*., 2024). Additionally, excessive glial cell activation may accompany DM, resulting in the production of copious quantities of inflammatory chemicals (Kumari *et al*., 2025). The natural *S. costus* extract contains a range of chemical compounds that not only inhibit histamine production but also possess anti-inflammatory and antioxidant properties. As a result, there is a decrease in pro-inflammatory cytokine levels (Elshaer *et al*., 2024).

The presence of Au-NPs in the nano-extract disrupts the transmission of inflammatory signals by interacting with IL-6 outside the cells, preventing it from attaching to cellular receptors. This interaction reduces the expression of TNF-α and IL-6-specific mRNAs, as well as inducible nitric oxide synthase, slowing the progression of inflammation (Zhang *et al*., 2024).

After fulfilling its purpose in preserving memory function, AChE hydrolyzes the neurotransmitter acetylcholine into choline and acetate, demonstrating its potent action in the cholinergic nervous system. Therefore, it is responsible for transmitting nerve signals and regulating synaptic communication (Gajendra *et al*., 2024).

It was found that the brains of the diabetic group had noticeably higher amounts of AChE and Aβ contents. This might indicate a direct correlation between the contents of AChE and Aβ. The increased AChE concentration in and around Aβ plaques is a result of the direct binding of increased Aβ to nicotinic receptors. Furthermore, once co-localized with Aβ deposits, AChE has the ability to create the Aβ-AChE complex, a highly toxic entity that further accelerates the aggregation of Aβ into amyloid fibrils (Stanciu *et al*., 2020)

After treatment with gold nano-extract, the Aβ concentration and AChE enzyme activity return to normal levels. This could be attributed to the hydrophobic characteristics of the enzyme environment in ChE molecules and the lipophilic nature of the Au-NPs. As a result, the inhibitory impact on AChE activity is more pronounced compared to the original extract. This may be due to the high affinity of Au-NPs for binding to ChEs and their primary interaction with the AChE protein to inhibit AChE activity (El Alami *et al*., 2020).

5. Conclusion

The hematological and biochemical measurements that were compromised in the STZ-induced diabetic rats were improved by treating them with native *S. costus* extract and gold nano-extract; only the gold nano-extract was able to return the values to normal. Both the native *S. costus* extract and gold nano-extract increased enzymatic and non-enzymatic antioxidants while reducing inflammatory

markers (TNF-α and IL-1β) and peroxidation reaction products (LPO and TPC) in the brain tissue homogenate, as measured. All measurement levels could only be normalized by the gold nano-extract.

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