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EXPLORING THE PROTECTIVE EFFECT OF IRRADIATED HIBISCUS AGAINST DIABETES INDUCTION IN ALBINO RATS

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ABSTRACT

Background: the treatment of diabetes has been the subject of several studies. Still, many have long wondered if there is a cost-effective approach to stop or significantly lower the number of cases of diabetes. Given that hibiscus has been linked to a wide range of therapeutic benefits, it was crucial to look into how effective it is as an antioxidant in reducing the prevalence of diabetes. **Aims:** The goal of the present study is to investigate whether irradiated hibiscus powder protects pancreatic cells from the damaging effects of streptozotocin (STZ). **Methods:** Twenty-one adult female albino rats were equally assigned into three groups. Group 1 served as a control group and received only water and a standard rat diet. Group 2 was diabetic and also received just water and a standard rat diet. Group 3 was diabetic and also received a standard rat diet plus water mixed with 10% irradiated hibiscus extract (v/v).Then after five weeks, Type 2 diabetes was induced in overnight fasted rats of G2 and G3 by STZ. At the end of the experiment, blood, liver, and pancreatic samples were collected for biochemical, molecular analyses as well as histological study. **Results:** Group 2 had significantly higher glucose levels after STZ induction compared to group 3. Moreover, there are significant increases in liver enzymes, inflammatory agent, cholesterol, and triglycerides as well as impaired liver functions in group 2 compared with others. Moreover, relative to group 3, there is a substantial reduction in molecular as well as histological damage compared with group 2. **In Conclusion:** For the first time, irradiated hibiscus, attracts attention to play a vital and cost effective role against diabetes incidence by STZ. This could give rise to the hope of using hibiscus as a safer, more cost-effective, and efficient beverage to decrease the spread of diabetes on a large scale.

KEYWORDS: diabetes, irradiated hibiscus, HIP-14, Caspase-3, liver functions, lipid profile, interleukin, postponing incidence of diabetes.

1. INTRODUCTION

Glucose intolerance is the hallmark symptom of diabetes, a metabolic disease that can last for years. Serious damage to the cardiovascular system, blood vessels, eyes, urinary system, as well as nerves can occur over time[1] Insulin resistance, poor insulin production by the β-cells of the Langerhans islets in the pancreas, and decreased insulin sensitivity of tissues (particularly skeletal muscles and the liver) are the hallmarks of Type 2 Diabetes Mellitus (T2DM).[2] In those with diabetes, it constitutes over 90% of cases.[3] Oral antidiuretic medication with a meal is the gold standard for therapy complex pharmacological effects. In many cases, using one antidiabetic medicine is not enough, and a combination of drugs is necessary.[4] One set of risk factors for type 2 diabetes, known as metabolic syndrome, is these conditions encompass, high blood pressure, central obesity, insulin resistance, as well as dyslipidemia.[5] Hyperglycemia is just one symptom of diabetes. It is a disease that can manifest in many different ways. Issues with lipid profiles, protein deficiency, and hypercholesterolemia can manifest in people with diabetes. In addition, high glucose level lead to diabetic retinopathy, kidney disease, nerve damage, ulcers, and inflammations.[6]

Moreover, an elevation in reactive oxygen species (ROS) generation, a byproduct of diabetes, causes oxidative stress. There is an oxidation of biological components caused by reactive oxygen species, this is the leading to most of complications and illnesses associated with diabetes.[7] The high cost of treating diabetes makes it helpful to discover new approaches that can supplement, improve, or replace the effects of synthetic anti-diabetic medications. The efficacy of herbal medications used to treat this illness has been documented in a number of studies.[8]

Egyptians cultivate hibiscus (roselle), a plant belonging to the Malvaceae family, is known for its many medicinal properties. The purple sepals (the calyx and epicalyx) and the plant's Egyptian name, "Karkadeh," constitute the plant's most valuable commercial components, serving as a natural food colourant in the cosmetics and jam & jelly sectors.[9]

According to a previous publication[10], the anthocyanin pigment, that are primarily responsible for the red color are cyanidin-3 glucoside and delphinidin 3 glucoside. Moreover, it has a good effect on gastric functions. It has a significant antiseptic function in the intestine and can be used against infections in the intestine, cancer, diabetes, obesity, and microorganisms, as well as a neuro-protective agent.[11]

Protocatechuic acid, is one of the basic phenolic components found in Hibiscus that may have anti-pyretic and anti-liver disease effects.[12] It has been shown that anthocyanin is a crucial factor in reducing diethylnitrosamine's carcinogenic effects in the liver.[13] Previous phytochemical studies have shown that this plant contains phenolic compounds, flavonols, anthocyanins as well as protocatechuic acid (PCA).[14] Most medicinal plants have significant levels of bioflavonoids and phenolic compounds, both of which have strong antioxidant abilities.[15]

Hibiscus is very common among diabetic patients, and their beliefs have been supported by in vitro and in vivo investigations.[16] The phytochemical components and therapeutic uses of this plant have been the subject of several investigations, but to date, no to further understanding on how Hibiscus protects against or at least reduces the development of diabetes caused by STZ.

Additionally, according an earlier publication[17], it was stated that gamma-irradiated hibiscus may be more beneficial than hibiscus alone in protecting against the oxidative stress caused by frequent frying oil exposure in vitro as well as in vivo tests. It might be the result of gamma irradiation, which raises the amount of total polyphenols as well as their antioxidant and angiotensin converting enzyme inhibitory action. Polyphenols are recognized to be the cause of the products' antioxidant activity.

Additionally, the increased total flavonoid and phenolic content under gamma irradiation may result from the compounds' liberation from their glycosidic forms, which could explain the better antioxidant benefits of irradiated hibiscus, in addition to the large compounds breaking down into smaller ones.[18] Thus, the present publication is devoted to this plant to explore whether irradiated hibiscus can be used as a protective, safer, and economic agent against diabetes incidence.

The importance of the current study: Current study may open the door to reducing or at least postponing the incidence of hereditary diabetes as well as gestational diabetes.

2. MATERIALS AND METHODS

2.1. MATERIALS: Mature, dried, dark-red hibiscus calyces were purchased from a neighborhood market in Cairo, Egypt. Every biochemical kite, such as the lipid profile include: triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL), interleukin-10 and 18, total antioxidant capacity, kidney functions include, urea, creatinine, and uric acid, as well as liver functions (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total protein were acquired from Linear Chemicals, S.L.U., Barcelona, Spain.

2.2. METHODS

2.2.1. Irradiation treatments: Using an experimental ^{60}Co gamma chamber (dose rate 665.6 Gy/h), the Cyclotron Project, Nuclear Research Center, Egyptian Atomic Energy Authority, Egypt, finely ground mature dry dark-red calyces of hibiscus plant were exposed to gamma irradiation at a dose of 1.5 KG/y.[17]

2.2.2. Preparation of Plant Extraction: After several trials, an amount of 250 g of dried powder of irradiated red hibiscus leaves was mixed with 1000 ml tap water and soaked for 24h, then filtered and mixed with tap drinking water by (10%v/v).

2.2.3. Experimental Induction of Type 2 Diabetes: Intraperitoneal injection of freshly produced STZ (60 mg/kg) mixed in 0.1 M cold citrate buffer, pH 4.5, was given to overnight fasted rats 15 minutes after intraperitoneal injection of 140 mg/kg nicotinamide, which was used to induce type 2 diabetes.[18]

2.2.4. Experimental animals: In the current study, twenty-one adult female albino rats (120-150g) were used. The experimental animals were kept in conventional laboratory conditions for two weeks during which they were permitted to acclimate to a room temperature of 28 ± 2 °C and a 12:12h light/dark cycle. The Research Ethics Committee of the National Center for Radiation Research and Technology, the Atomic Energy Authority Egypt, approved the experimental protocol with serial number 87A/23 for monitoring and supervising experimental animals.

2.3. Experimental Design: The rats were divided into three equal groups, seven rats each: **Control group (G1)**; healthy and untreated rats served as a normal control and maintained on regular rat diet and water only. **Diabetic group (G2)**; fed with regular rat diet and water. **Diabetic + Irradiated Hibiscus group (G3)**; fed with regular rat diet and water mixed with 10% irradiated hibiscus extract (10%v/v). Rats were fed for 5 weeks,

then, type2 diabetes was induced by STZ (60 mg/kg b. wt.), 15 minutes after intra-peritoneal administration of 140 mg/kg b. wt. Nicotinamide in G2 and G3. After72h, blood glucose level was measured immediately using blood glucose meter.

2.4. Blood and tissue sampling: At the end of the experiment (6 weeks), rats were sacrificed by decapitation Blood samples were taken for Complete blood count as well as biochemical analysis. Serum was obtained after centrifugation at 3,000 rpm for 15 min and then kept at -20°C for further biochemical studies.[19] Then, the liver, kidney and pancreas specimens were quickly removed and washed for (molecular and histopathological studies).

2.5. Hematological and biochemical Analysis: The blood glucose meter was used to measure the blood glucose level right away. A complete blood cell counter (Abacues 380 CBC counter) was used to measure the CBC (Hb%, WBC, RBCs, and platelets). The parameters that were assessed included serum concentrations of total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TG), bilirubin, total protein, albumin, urea, creatinine and uric acid, total antioxidant, and interleukin 10&18, these were all measured in accordance with the manufacturer's instructions for Bio-Kinetic methods provided by Linear Chemicals, S.L.U., Barcelona, Spain.

2.6. RNA Isolation and Quantitative Real-Time PCR (qRT-PCR) for Caspase-3 and HIP-14 gene expression: Kidney and pancreas specimens were quickly removed and washed for molecular studies. RNA extraction from tissue samples was applied using QI Aamp R Neasy Mini kit (Qiagen, Germany, GmbH), when 200 µl of the sample were added to 600 µl RLT buffer containing 10µl β-mercaptoethanol per 1 ml, then incubated at room temperature for 10 min. One volume of 70% ethanol was added to the cleared lysate, and the steps were completed according to the purification of Total RNA protocol of the QI Aamp R Neasy Mini kit (Qiagen, Germany, GmbH). N. B. On column D Nase digestion was conducted to remove the residual of DNA. Primers used were supplied from **Metabion (Germany)** (Table 1). Real-time PCR (qPCR) was carried out using the reaction mixture of a 10 µl of the 2x HERA SYBR® Green RT-qPCR Master Mix (Willow fort, UK), 1 µl of RT Enzyme Mix (20X), 1 µl of each primer of 20 pmol concentration, 3 µl of water, and 5 µl of RNA template in a total volume of 20 µl. Under the exact parameters listed in Table (1), the reaction was carried out in a step one real-time PCR. The step one software produced amplification curves and ct values. Using the ratio (2-DDct), the CT of each sample was compared with that of the positive control group using the "ΔΔCt" approach as described by Yuan et al., 2006[20] in order to determine the variation of gene expression on the RNA of the different samples.

Table (1): Primers sequences, target genes, amplicon sizes, and cycling conditions for SYBR green rt-PCR.

Target gene	Primers sequences	Reverse transcription	Primary Denaturation	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	
B- actin	CCTGCTTGCTGATCCACA	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	60˚C 30 sec.	72˚C 30 sec.	21
	CTGACCGAGCGTGGCTAG						
Caspase-3	GTGGAACTGACGATGATATGGC						22
	CGCAAAGTGACTGGATGAACC						
HIP-14	TACCGAAGCGGGCTGTGT						23
	AGTTTTCCGTCCAAGAGGTTCAC						

2.7. Histopathological study: Rats were killed, their livers were removed, and histological investigations were conducted on them to highlight the existence of liver damage. The livers underwent a series of procedures including washing, fixing with 10% formalin (pH 7.4), paraffin immersion, cutting at 5-micrometer intervals, and staining the slides with hematoxylin and eosin. After observing the pathological alterations on tissue slides by optical microscopy, pictures were captured at H&E X200.[24]

2.8. Statistical analysis: Using SPSS software version 16, one-way ANOVA was used to statistically examine the study's data, and then the post hoc Duncan's test was performed. When $P < 0.05$, the values were deemed significant.[25] Moreover, GraphPad Prism8 software (version 8.00, GraphPad program, Inc., La Jolla, CA, USA) was used to design the graphs.

3. RESULTS

Data of the present study showed a highly significant increase in glucose level in "G2" after 72h of diabetes induction by STZ, compared with G1. Meanwhile there is no any significant increase in glucose level in G3 when compared with G2 as well as G1 as shown in Table (2). In addition, induction of diabetes by STZ, induced significant hazardous effects in biochemical parameters, as well as molecular and histological studies. Meanwhile, hibiscus administration at a dose of 10% v/v (hibiscus extract was mixed with tap water), protected and decreased the biochemical, molecular, and histological hazardous effects of STZ which will be covered in details on (molecular, hematological, biochemical and histological level).

3.1. Molecular studies include Relative mRNA expression of Caspase-3 and HIP-14: To underline the cellular mechanism of STZ influence on the pancreas and kidney, mRNA expression of apoptotic (Caspase-3)

and anti-apoptotic (HIP-14), related genes were evaluated by qRT-PCR. The current study found that Caspase-3 mRNA gene expression was significantly up-regulated in G2 compared with G1. Meanwhile, it was down regulated again in G3 compared with G2. Contrarily, it was found that in G2, HIP-14 mRNA gene expression showed a highly significant reduction in comparison with G1, interestingly its expression level in G3 was significantly raised relative to G2 in both kidney and pancreas tissues, as shown in Fig. (1).

3.2. **Hematological studies including complete blood count:** The current results showed that there are significant decreases in hemoglobin and red blood corpuscles in G2 compared with G1, while administration of irradiated hibiscus in G3 ameliorated the harmful effects of STZ as shown in Table (3).

3.3. Biochemical studies

A- Liver Function tests: The current study's data suggested that STZ had a negative impact on liver enzymes because levels of AlT, AST, and ALP in G2 significantly increased when compared to G1 and G1. Additionally, compared to G1, there is a notable drop in albumin and total protein in G2. In the meantime, the G3's liver enzyme levels (ALT, AST, and ALP) are much lower than those of the G2 group. Furthermore, Table (4) reveals that the levels of albumin and total protein in G3 are normal in comparison with G2.

B- Kidney function tests: The current data found a significant increase in urea, creatinine, and uric acid levels in G2 compared with G1 by percentage of changes of 125, 30, 39 %, respectively, indicating kidney damage. Interestingly, pretreatment with irradiated hibiscus G3 could protect the kidney function through keeping the normal levels of renal markers after Diabetes induction by STZ as decreased this hazards effects by percent of change, -33, -22.7, -14.35% respectively.

C- Total antioxidant capacity (TAC): Furthermore, this study demonstrated that the TAC level in (G2) increased significantly by 563% as a percentage of change when compared to (G1). Meanwhile, there was a significant drop in TAC level in (G3) as compared to (G2) by -65.85% as shown in Table (6).

D- Lipid profile: According to the obtained findings, G2 has much higher levels of LDL, VLDL, triglycerides, cholesterol, than G1. Table (6) illustrates how G3's levels of cholesterol, triglycerides, LDL, and VLDL decreased significantly in comparison to G2 by percentage changes of -24, -21.6, -32.5, and 28%.

E- Interleukins 10 and 18: The current data showed highly significant increases in IL18 and highly significant decreases in IL10 in G2, compared with G1, meanwhile G3 showed a significant reduce in IL18 and significant elevation in IL10 compared with G2 by percentage changes of -45 and 171% respectively as shown in Table (7).

3.4. Histopathological Investigations: The biochemical analysis in each of the study groups was validated by histological analysis using Hematoxylin and Eosin stains on the liver and pancreatic tissues, as illustrated in Fig. (2).The liver from "G1" displayed normal blood sinusoids, normal central vein, and normal polygonal hepatocytes in the hepatic parenchyma (Figure. 3A). However, the liver from "G2" (arrow head) shows extensive vacuolar degeneration and a congested central vein with hepatocytes, the liver from "G3" (Fig. 3C) appears to have normal hepatocytes and neither vacuolar degeneration nor central vein congestion.

Moreover, in pancreas tissue, Pancreas from “G1” exhibited normal pancreatic tissue; the normal pancreatic acini with no signs of inflammation (Fig. 4A) was noted, while in “G2”, Pancreas from Diabetic group exhibited severely dilated blood vessels (arrow head) with thick muscle wall (arrow), together with necrosis pancreatic acini (Fig. 4B). Contrarily, Pancreas from "G3" group (Fig. 4C) showed apparently normal pancreatic acini with absence of the necrosis and moderate regression of the blood vessel congestion (H&E X200).

Table (2): Effects of different treatments on the glucose levels before and after diabetes induction in all groups.

Groups		(Glucose before induction (mg/dl)	(Glucose after induction mg/dl
Control "G1"	Mean ±SD	113±13	120±4
	%change	-----	-----
Diabetic group "G2"	Mean ±SD	106±9	474±25$^{###}$
	%change	-6%	295%
Hibiscus group "G3"	Mean ±SD	102±8	143±44***
	%change	-3%	-69.81%

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the adverse effects of STZ compared with (G2) represent the change percentage.

Table (3): Effects of different treatments on complete blood count in all groups.

Groups		Hb% (g/dl)	RBC m.c\cmm	WBC ×103 /cmm	PLT ×103 /cmm
Control "G1"	Mean ± SD	14.6 ± 0.4	4.8 ± 0.83	7.61 ± 0.67	741 ± 55
	% change	-----	-----	-----	-----
Diabetic group "G2"	Mean ± SD	9.6 ± 0.7##	2.69 ± 0.2#	11.07 ± 2.6#	330 ± 100##
	% change	-34.2%	-43.9%	45%	-124.5%
Diabetic + Irradiated Hibiscus group "G3"	Mean ± SD	13.2 ± 0.9*	4.2 ± 0.4*	9.32 ± 0.59	410 ± 89*
	% change	37.5%	56.1%	-15.8%	24.2 %

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the adverse effects of STZ compared with (G2) represent the change percentage.

Table (4): Effects of different treatments on Liver functions in all groups.

Groups		Total P. "g/dl"	Albumin "g/dl"	ALP (U/l)	AST (U/l)	ALT (U/l)
Control "G1"	Mean ±SD	7.2±0.32	3.78±0.14	139±13	58±8	55.6±8
	% change	-----	-----	-----	-----	-----
Diabetic group "G2"	Mean ±SD	5.86±0.3##	3.67±0.14	301±19.7#	209±18##	141±8##
	%change	-18.6%	-2.9%	116.5%	260.3%	153%
Diabetic + Irradiated Hibiscus group "G3"	Mean ±SD	6.75±0.23*	3.81±0.31	208±32**	128±22.8**	96±8**
	%change	15.2%	3.8%	-30.89%	-38.75 %	-31.9 %

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the adverse effects of STZ compared with (G2) represent the change percentage.

Table (5): Effect of different treatments on kidney functions in all groups.

Groups		Urea (mg/dl)	Creat. (mg/dl)	Uric acid (mg/dl)
Control "G1"group	Mean ±SD	22.3± 3.5	0.84±0.06	4.36 ± 0 .26
	% change	-----	-----	-----
Diabetic group "G2"	Mean ±SD	50.3±1.5##	1.1 ±0.08##	6.06 ±0.26#
	%change	125.5 %	30.9%	39 %
Diabetic + Irradiated Hibiscus group "G3	Mean ±SD	33.6 ±3.5 **	0.85± 0.06**	5.19 ±0.33*
	%change	-33.2%	- 22.7%	-14.35 %

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the adverse effects of STZ compared with (G2) represent the change percentage.

Table (6): Effects of different treatments on Lipid profile, and Total antioxidant capacity (TAC) in all groups.

Groups		Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TAC (mM/L)
Control "G1"	Mean ±SD	103±17	94.3±8.3	45.3±2	39.3±16	19±1.7	0.94 ±0.08
	%change	-----	-----	-----	-----	-----	-----
Diabetic group "G2"	Mean ±SD	208±23##	157.3±8.7#	45.6±3	132±28##	32.6±2.5##	6.24 ±1.46#3
	%change	101.9%	67%	0.66%	238.4%	68.4%	563 %
Diabetic + Irradiated Hibiscus group "G3"	Mean ±SD	158±8*	123.3±17.9*	45±5	89±11.7*	23.3±2*	2.13 ±0.25 **
	% change	-24%	-21.6%	-1.3%	-32.57 %	-28.1 %	-65.85%

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the

adverse effects of STZ compared with (G2) represent the change percentage.

Table (7): Effects of different treatments on Interleukin 10&18 in all groups.

Groups		IL 18 (pg/mL)	IL 10 (pg/mL)
Control "G1"	Mean ±SD	249±26	870±75
	%change	-----	-----
Diabetic group "G2"	Mean ±SD	737±56$^{###}$	212±32$^{###}$
	%change	195%	-75%
Hibiscus group "G3"	Mean ±SD	402±43***	575±91***
	% change	-45%	171%

The mean ± SD was used to express numerical data. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with normal control group (G1) (#). A P value of less than 0.05 is deemed significant. The damaging effects of STZ on (G2) compared with (G1) and the protective effect of irradiation hibiscus (G3) against the adverse effects of STZ compared with (G2) represent the change percentage.

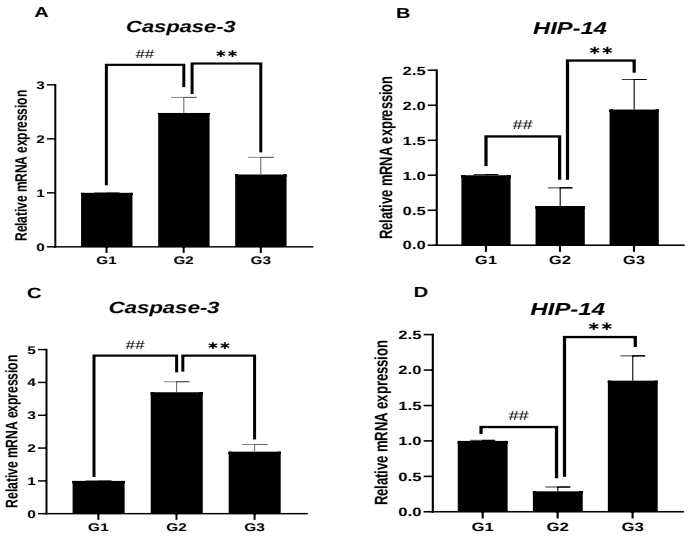

Figure (1): Effects of various treatments on HIP-14 and Caspase-3 across all groups Caspase-3 and HIP-14 mRNA expression in the kidney is shown in (A, B), and in the pancreas it is shown in (C, D). The mean ± SD of the results is displayed. Diabetic + Irradiated Hibiscus (G3) group is compared with Diabetic group (G2) (*), and Diabetic group (G2) is compared with the normal control group (G1) (#). P < 0.05 is regarded as significant.

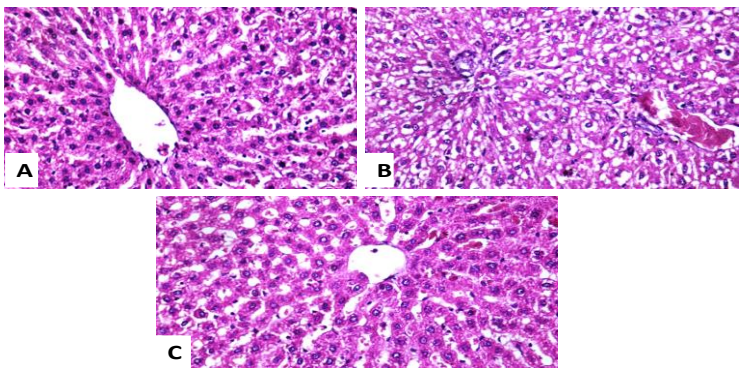

Figure (2): (A, B, and C) showing control group "G1", "A" Liver from Control group postulated normal liver parenchyma; as the normal polygonal hepatocytes, normal central vein, as well as normal blood sinusoids), in the other hand "B", showing Liver from Diabetic group showing congested central vein (arrow head) and severe vacuolar degenerated hepatocytes, meanwhile, in G3, Liver from irradiated hibiscus group showing apparently normal hepatocytes with no vacuolar degeneration nor congestion of the central vein

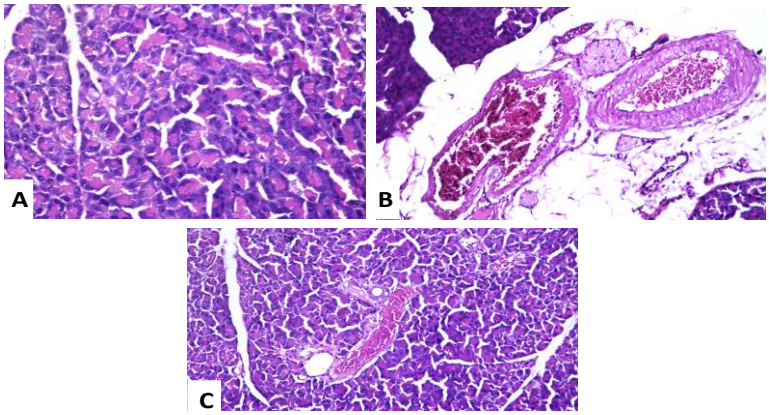

Figure (3): (A, B, and C) shows pancreas tissue, Pancreas from Control group shows normal pancreatic tissue; note the normal pancreatic acini with no signs of inflammation, while in G2 Pancreas from Diabetic group shows severely dilated blood vessels (arrowhead) with thick muscle wall (arrow), together with necrosis pancreatic acini, while, Pancreas from hibiscus"G3" group showing apparently normal pancreatic acini with absence of the necrosis and moderate regression of the blood vessel congestion (H&E X200)

4. DISCUSSION

While most studies aim to find different treatments for diabetes, the current study aimed to find a new treatment to prevent or at least decrease the incidence of diabetes induced by STZ. Indeed, the current study postulated that irradiated hibiscus works to protect pancreatic cell against diabetes incidence in female albino rats. The authors will shed light on the mechanism of irradiated hibiscus in preventing the incidence of diabetes by STZ.
First of all, the data of total antioxidant capacity (TAC) should be discussed because it is the key player in the current data and its explanations.

Redox equilibrium is essential for cells and the organism as a whole, according to strong evidence: when it is disturbed, nearly every biological activity can be affected, including metabolism, cell proliferation, differentiation, cellular senescence, and autophagy.[26] Crucially, "oxidant" refers to a broad category that includes several different types of oxidants formed from molecular oxygen, and the overall name for this family is reactive oxygen species (ROS).[27] In order to maintain redox balance and prevent harm, antioxidant defenses routinely deactivate this physiological oxidant generation. Oxidants can cause harm to an organism if its antioxidant defenses are weak or if redox signaling is disrupted. Oxidants can, for instance, damage cell membranes and inhibit the activity of important enzymes and processes, leading to hazards effects.

The data of the present study demonstrated that as compared to the diabetic group, the levels of total antioxidant capacity (TAC) in the normal and irradiated hibiscus groups were significantly lower. When the TAC value is low, it can mean that one's health is in normal clinical state, and not responding well to the rising radical generation, or that a person uses many antioxidants for being under high oxidative stress. Thus, a subject's antioxidant status might be better understood by combining TAC examination with other tests of particular antioxidants and indicators of oxidative damage.

Because the present data picture shows that, in comparison to the normal and irradiated hibiscus groups, the diabetes group has elevated glucose levels and hazardous impacts on biological functioning. Therefore, a stable clinical status may have been indicated by the lower value of TAC in the irradiated hibiscus group compared to the diabetic group. Consistent with this finding is the study of SAVU et al., 2012[28], who postulated increased levels of oxidative stress, despite higher antioxidant capacity, in the plasma of patients with uncomplicated type 2 diabetes compared with healthy control subjects.

Indeed, irradiated hibiscus is able to protect cells from oxidative stress of STZ, it may be related to significant increases in oxygen consumption in irradiated hibiscus group compared with oxidative stress group[17], who showed highly effective role of irradiated hibiscus as antioxidant agent.

The authors would contest that despite the widespread evidence suggesting a positive correlation between rising oxygen consumption and rising reactive oxygen species production, this relationship would only hold if rising oxygen consumption was a result of elevated tissue pO2 or an increase in the quantity of "sites," or functional mitochondria. On the other hand, a rise in oxygen consumption under conditions of fixed mitochondrial number and constant tissue oxygen concentration would support a fall in ROS levels.[29]

Furthermore, the idea that reducing ROS production through increased mitochondrial ATP generation efficiency has given rise to the "uncoupling to survive" hypothesis.[30] A recent study[31] provides support for this hypothesis by showing that in an outbred strain of mice, animals with higher metabolic intensities and oxygen

consumption survived longer than those with lower metabolic intent+sites. These long-lived, high-metabolism rate mice also exhibited large increases in the degree of metabolic uncoupling, indicating that by lowering the mitochondrial membrane potential, these animals may be able to limit ROS formation even in the presence of elevated oxygen consumption.[29]

Furthermore, this view aligns with the findings of Wali et al.[32], who demonstrated that BIM− /− cells exhibited a greater rate of mitochondrial oxygen consumption, which was linked to increased mitochondrial complex IV activity. Increased oxygen consumption in BIM−/− mice resulted in markedly decreased body weights, decreased adiposity, and decreased hepatic lipid content. Reduced adiposity was correlated with lower fasting blood glucose, increased insulin sensitivity, and improved hepatic insulin signaling in BIM−/− mice.

Therefore, the authors attempt to explain the data of the current study by taking main two points namely, the oxidative effect of STZ, and the antioxidant effect of irradiated hibiscus, which protected or at least reduced the oxidative stress damaging of STZ in (molecular, hematological, biochemical and histological levels).

Expression of both Caspase-3 and HIP-14 genes was evaluated in pancreas and kidney to study the anti-apoptotic properties of hibiscus. Caspase-3 has been widely studied in numerous tissues due to its role as the fundamental executioner of apoptosis.[33] Huntingtin-interacting protein 14 (HIP-14) has anti-apoptotic properties for β-cells and provides a significant effect in glucose-stimulated insulin production.[34] In the present study, it was shown that Caspase-3 was up- regulated and HIP-14 was down regulated in the G2 compared with G1. Meanwhile, Caspase-3 was down regulated, and HIP-14 was up-regulated in G3 compared with the G2. It is interesting to note that research on type 1 diabetes rats given STZ to induce diabetes also suggested that the flavonoid-rich aqueous fraction of a methanolic extract of hibiscus calyces would have comparable effects. The current study showed a rise in the volume and quantity of β-cells in the pancreatic islets. The antioxidant properties of hibiscus most likely account for this capacity to preserve and repair pancreatic islets.[35] Furthermore, the methanolic extract of Hibiscus was found to have the ability to influence the glucagon-like peptide-1 (GLP-1) hormone.[36] This hormone, which is generated in the ileum, is important for the pancreas because it increases insulin secretion, promotes cell proliferation, and inhibits the death of pancreatic β cells.[37]

Since the release of pro-inflammatory cytokines is consistently linked to hyperglycemia, both oxidative stress and inflammation are important factors in diabetes.[38,39] Virgolici et al.[40] reported that there was a correlation between higher oxidative stress and higher levels of inflammation. According to some theories, inflammation is the host's defensive response to infections as well as tissue injury. It can promote tissue repair or stop the spread of pathogens.[41,42] Cytokines trigger an immunological response in diabetes by activating NF-κB, which leads to pancreatic β-cell death. Strong pro-inflammatory cytokine interleukin-18 (IL-18) regulates both innate and acquired immune responses and aids in the host's defense against infections.[43] One of the main anti-inflammatory cytokines, interleukin 10 (IL10), has the ability to inhibit pro-inflammatory reactions in both innate and adaptive immune cells. The current investigation revealed that in comparison to G1, G2 had highly significant increases in IL18 and highly significant decreases in IL10; in contrast, the irradiated hibiscus group G3 demonstrated large increases in IL10 and decreases in IL18. The hibiscus extract's antioxidant concentration prevents or at least lessens inflammation. The obtained results were also in line with earlier research on the anti-inflammatory benefits of H. sabdariffa in diabetic animal models, where it was shown that phenol and flavonoids were significant pro-inflammatory cytokine modulators.[44,45]

The current findings showed that the diabetic group had higher white blood cell counts than the control group, while having significant reductions in Hb% concentration, red blood cell count, and platelet count. This decrease may be related to both the inflammatory and oxidative stress effects of STZ. Moreover, the increased glycosylation of various proteins, including hemoglobin, in diabetes may provide an additional explanation for this.[46] Nonetheless, the hematological parameters were improved by pretreatment with irradiation hibiscus. The antioxidant activity of the hibiscus extract may be the cause of this improvement.

Rats with diabetes were also shown to have lower serum total protein levels, which is in line with the findings of an earlier publication.[47] Microproteinuria, one of the most important clinical markers of diabetic nephropathy[48,49], and enhanced protein catabolism could be the cause of the protein decrease. The pretreatment of diabetic rats with irradiated hibiscus generated a considerable normal total protein and level as compared with their normal levels, according to the study's results. Rats with experimental diabetes were shown to have an improvement in blood protein following oral treatment of Hibiscus rosasinensis.[47] Because these enzymes appear to have spilled from the liver into the bloodstream, elevated levels of liver enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase) in the bloodstream postulate hepatocellular damage.[50] The present findings demonstrated that the activities of AST, ALT, and ALP were significantly greater in diabetic rats, which is consistent with other data.[51-53] However, the G3 rats' AST, ALT, and ALP activities were unaffected by the oral pre-administration of irradiated hibiscus. These results proved that the extract of Hibiscus anthocyanins had hepatoprotective qualities.

Measurements were made of urea, uric acid, and creatinine because diabetic kidney disease (DM) also damages kidney tissue because of abnormal glucose regulation, which results in elevated levels of glucose and glycosylated protein tissue, changes in kidney tissue hemodynamics, and elevation of oxidative stress.[54] The STZ-induced diabetes rats showed noticeably elevated levels of creatinine, uric acid, and plasma urea in comparison to the control group, suggesting that diabetic animals have impaired renal function.[55]

Nevertheless, hibiscus extract protected the kidney function, which is normally compromised in diabetic rats, bringing these plasma values down to a control range. In their research using a rat model of STZ diabetes, Wang et al.[56], discovered that feeding hibiscus flower aqueous extracts for eight weeks can lessen the symptoms of diabetic nephropathy. However, hibiscus reduces hyperglycemia, which has been linked to increased levels of reactive oxygen species and oxidative stress. This was discovered in a previous study. They also proposed that hibiscus can lessen lipid peroxidation in the kidneys of rats by significantly increasing the activity of catalase and glutathione levels in these organs.[57]

According to the current study's hypothesis, which is in line with other research[58-60], the diabetic group showed significantly higher levels of LDL, triglycerides, and total cholesterol when compared to the control group. This is because oxidative stress in the tissues is brought on by reactive oxygen species (ROS).[61] Conversely, the administration of irradiated hibiscus decreased the levels of TG, LDL, and TC. Numerous studies on hibiscus have identified flavonoids, polyphenols, phytosterols, and phenolic components—all of which are known to possess antioxidant qualities.[62] Antioxidants have been shown in numerous studies to protect biological tissues from oxidative stress and to prevent a number of human diseases.[63] Furthermore, hibiscus' anthocyanins have been shown to possess several times more antioxidant activity than ascorbate and other popular antioxidants.[64]

To confirm both molecular and biochemical studies, the histological study of both liver and pancreas tissues was conducted. It has been found that there are very hazardous effects on both liver and pancreas tissues in diabetes group. These results are consistent with those of other researcher[47], who demonstrated that, in contrast to necrosis and apoptosis of a small number of hepatocytes, the liver of diabetic rats treated with STZ had 2-3 foci of interlobular lymphocytes predominating inflammatory cell infiltration. Furthermore, the current findings aligned with those reported by Muhammadi et al[65], who demonstrated that the use of STZ to induce diabetes resulted in the destruction of beta cells with many nuclear vacuolization and a dilated rough endoplasmic reticulum. Moreover, beta cells had smaller pyknotic nuclei, a serrated nuclear membrane with portions of the cytoplasm that were electron-translucent, and fewer insulin secretory granules. Conversely, irradiated hibiscus can shield pancreatic and liver tissues from the oxidative damage caused by STZ.

Collectively, the current overall results concluded that hibiscus exposed to gamma radiation might be involved in reducing the oxidative stress that STZ experiences. This may be associated with gamma irradiation, which increases antioxidant activity, angiotensin converting enzyme (ACE) inhibitory effect, and total polyphenol concentration. It has been discovered that the goods' antioxidant properties are due to polyphenols. By contributing electrons to the 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) radical, their ability to scavenge free radicals enhances the radical's antioxidant activity.[69]

5. CONCLUSION

The data of current study open the door for researchers to clarify three basic points, i, irradiated hibiscus is a powerful protective agent against diabetes incidence, ii, irradiated hibiscus as antioxidant agent reduces hazardous effects of STZ on biological functions, iii, there are concept of using different treatments for diabetes with its potential for harmful effects on human health. On the other hand, irradiated hibiscus has the potential to reduce the occurrence of diabetes while also being a safe and cost-effective agent. This might enable us to combat these negative consequences in the upcoming years while also accounting for the patient's financial expenses.

ABBREVIATIONS

STZ	**Streptozotocin**
T2DM	Type2 diabetes
PCA	Protocatechuic acid
TAC	Total antioxidant capacity
IL18 &10	Interleukin 18&10
BIM	Building information modeling
GLP-1	Glucagon-like peptide-1
ACE	Angiotensin converting enzyme
DPPH	Diphenyl-1-picrylhydrazyl radical

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Conflict of interest, all authors declare that they have no conflict of interest.
Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.
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Ethical approval: All procedures performed in studies were in accordance with the ethical standards of the institutional and national research committee.

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