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Research Article

A COMPARATIVE STUDY ON COMMERCIAL BARBALOIN AND ISOLATED BARBALOIN FROM *ALOE VERA* FOR *IN VIVO* ANTI-DIABETIC AND ANTI-CANCER ACTIVITY

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ABSTRACT

Objectives: The fundamental objective of this research is to provide a thorough assessment of the anti-diabetic and anti-cancer properties of both commercial and isolated barbaloin obtained from *Aloe vera*. The study examines the usefulness of barbaloin, a significant secondary metabolite, in alleviating difficulties related to diabetes and cancer, in light of the growing worldwide interest in plant-based, non-toxic treatments.

Methods: The structure elucidation of the isolated molecule was performed using Fourier transform infrared, high-resolution mass spectrometry, and NMR. The experimental strategy incorporates *in vivo* experiments using streptozotocin-induced diabetic rats and Ehrlich ascites carcinoma (EAC) tumor models. Diabetic rats received varying dosages of isolated and commercial barbaloin, in conjunction with the common anti-diabetic medication glyburide, to evaluate their effects on blood glucose levels, serum biomarkers, and lipid profiles over 28-day duration.

Results: The findings indicated substantial decreases in blood glucose, serum enzymes, and lipid levels in the barbaloin-treated groups relative to untreated diabetic controls, indicating strong anti-hyperglycemic efficacy. Biochemical tests indicated enhancements in liver function indicators and oxidative stress parameters, corroborating the therapeutic efficacy of barbaloin in mitigating diabetes consequences. Simultaneous anti-cancer studies included the injection of EAC cells into rats, followed by treatment with different dosages of *A. vera* extract and barbaloin. The results indicated a significant reduction in tumor burden and improved survival rates, suggesting that barbaloin has substantial cytotoxic properties against cancer cells. The standard barbaloin reduced tumor volume by 85.4% and viable tumor cell count by 92.3%, isolated barbaloin reduced these parameters by 72.1% and 82.6%, respectively.

Conclusion: The research emphasizes the prospective therapeutic potential of barbaloin in the management of diabetes and cancer, indicating its viability as a safe, plant-based alternative or complement to traditional therapies.

Keywords: Ehrlich ascites carcinoma, Hyperglycemia, Cancer, Traditional medicine.

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INTRODUCTION

Diabetes is characterized by persistently elevated blood glucose levels. Diabetes ultimately compromises the heart, blood vessels, eyes, kidneys, and nerves. Adults are mostly susceptible to developing type 2 diabetes, a condition that arises when the body ceases insulin production or becomes resistant to its effects. Type 2 diabetes has proliferated throughout all socioeconomic strata during the past 30 years. The illness directly accounts for 1.5 million fatalities per year and impacts 422 million people worldwide, mostly in low- and middle-income countries. The role of protein glycation in the occurrence of complications with a number of diabetes-related sequelae, such as vasculopathy, retinopathy, nephropathy, neuropathy, cataracts, and chronic kidney disease, is accepted [1]. The consequence of this is glycation of products that lead to the production of advanced glycation end-products (AGEs) that can compromise protein integrity and hinder enzyme activities. AGEs are produced in a non-enzymatic, Maillard reaction, between the carbonyl group of a sugar or reducing aldehyde and the free amino-group of a protein. Initial production of Schiff bases is the first phase of the process, followed by other chemical transformations to yield both luminescent AGEs (such as pentosidine) and non-luminescent forms, e.g., carboxymethyl lysine (CML). Other markers of glycoxidative stress that has increasingly become reliable include compounds such as CML and pentosidine [5]. Since glycation has a significant role to play in diabetic complications [3,4], coming up with efficient protein glycation-inhibiting agents has become a major therapeutic goal in reducing or preventing the progression of diabetes related diseases [2]. In many developing nations, cancer is a major health issue and the second cause of death in the world at large [6]. This disease is non-specific and more common in older people due to several years of life that expose the individual to further damage to the DNA. According to the epidemiological evidence, men are more affected by cancer of the lungs, the colon and the rectum, and prostate, whereas women are more and more affected by breast cancer, colon and rectum cancer, and gastric cancer [7]. Surgery, radiotherapy, chemotherapy, immunotherapy, and diverse pharmacological methods are widespread in the treatment of cancer [8]. Localized cancer cells are usually treated using surgery and radiation to prevent the spread of cancerous cells to other parts of the body. The interventions are physically straining and can hurt the overall health of the patients. Many antineoplastic agents often harm proliferating normal cells and malignant ones [9]. Recent studies involve finding new anticancer agents targeting the tumor cells so as to minimize the damage to the normal tissues.

The use of herbal medicine dates back hundreds of years in an attempt to treat health complications. The fact that medicinal plants have minor side effects has made them remain vital therapeutic alternatives in developing countries. It was found out that about 20% of the drugs used worldwide start in plant life [10]. With increasing interest around the world in non-toxic and safe botanical substitutes in treating and preventing chronic conditions such as diabetes and cancer, there is a growing body of interest in the management of these and other chronic conditions using botanical agents. The *Aloe vera* (L.) Burm.f. is more than 5000 years old in a variety of cultures, such as Egyptian, Indian,

Chinese, and European, owing to its important healing and treatment capabilities [11,12]. *A. vera* is a genus that has about 450 species and is very adaptable in arid, tropical, and subtropical areas. It is a succulent growth and may or may not have a stem, but what it has almost always has is a stem that does not exceed 60–100 cm in height. It has thick and fleshy triangular leaves with spiny edges, which resemble the leaves of a cactus, although placed in the Liliaceae family. The water-holding ability of the plant can survive long droughts, when most of the other plant species perish [13]. The *A. vera* contains a lot of compounds, including barbaloin, vitamins, minerals, enzymes, polysaccharides, phenolics, and organic acids, among others (more than 70).

Barbaloin (10-beta-D-glucopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9[10H]anthracenone) is a main secondary metabolite, widely distributed around the world, that exists in Aloe plants, mostly of this particular genus. Yet another characteristic of barbaloin is its generation of yellow fluorescence. It has been shown that the concentration level is higher in the living aloe leaves, which is more concentrated in the terminal one-third, than in the older leaves [14]. Barbaloin – it is a derivative of C-glycoside of aloe-emodin anthrone and is largely found in the outer rind of the plant. Barbaloin is absorbed very poorly when taken in the oral route; instead, barbaloin is broken down by the intestinal microflora into aloe-emodin, which is more efficiently absorbed. Barbaloin and aloe-emodin are known to be laxatives and can sometimes be applied as bittering agents in alcoholic beverages. In addition to its cathartic and anti-inflammatory properties demonstrated *in vivo, in vitro* studies have also demonstrated that barbaloin is selective to cancer cells with strong inhibitory hepatic stellate cells activation [15].

This study focused on the potential antidiabetic and anticancer activity of isolated barbaloin and evaluated the efficacy of isolated barbaloin. Comparison with commercial barbaloin and isolated barbaloin provides insight into the potency of the isolated product.

METHODS

Collection of samples

The *A. vera* plant is collected from the local area of Bira, West Bengal, India (Lat 22°.80'N Long 88°.55'E). Standard barbaloin, streptozotocin (STZ), and 5-fluorouracil (5-FU) are purchased from Spectrochem Pvt. Ltd, Mumbai; the glibenclamide was procured from Hoechst India. The remaining reagents were all purchased from commercial sources and were of analytical quality.

Extraction of phytoconstituents

Rinsed the *A. vera* leaves with water and excised the rinds. We extracted the gel from the inside, fractured it into bits, and dehydrated it using a conventional hot air drier at 60°C with a consistent air velocity of 1.5 m/s. The desiccated substance was then pulverized. The desiccated coarse powdered *A. vera* gels (200 g) were first defatted using petroleum ether (60–80°C) and then extracted with 500 mL of 90% ethanol in a Soxhlet apparatus (LABTRON) [16].

Isolation of barbaloin from the extract using column chromatography

The ethanol extract was sequentially partitioned between chloroform and ethyl acetate (3 × 1 L). The respective solvents were similarly eliminated at decreased pressure, yielding an ethyl acetate fraction (E.A.F.) of 50 g and a chloroform fraction. 7 g of the E.A.F. were adsorbed onto silica gel (60 G, Merck, 600 g) and then put into a silica gel column. A gradient of n-butanol, acetic acid, and water in a 4:1:1 ratio was used to elute the column, resulting in the collection of 100 fractions, each measuring 50 mL. Fractions 35–42 were amalgamated, and thin-layer chromatography (TLC) revealed a singular spot with an R_f value of 0.58. The mixed fractions were evaporated to dryness (Rotary evaporator; RV 8V-C Digital) and then rechromatographed on a silica gel column using gradient elution with chloroform: ethyl acetate (8:2), yielding a single product that was recrystallized with methanol to produce a pure yellowish-brown powder (Barbaloin).

Determination of barbaloin using TLC (Fig. 8)

The plates are manufactured using silica gel, with a thick coating of the silica gel mixture applied on the plates, maintaining a slurry thickness of around 1–1.5 mm. After preparation, the plates were dried for 30 min in a hot air oven at 110°C. The melting point capillary is used to create a spot. The capillaries were filled with a dilute solution, and the solvent will facilitate the evaporation of the drug solution, allowing it to reappear in the same place. A tiny or focused area is applied with this procedure. The TLC chamber (Z266019, Sigma-Aldrich) used a solvent solution of ethyl acetate, methanol, and water at a ratio of 100:13.5:10. The prepared plates were positioned in the TLC chamber, which was thereafter sealed with a cover. The plates will facilitate the creation of spots, after which they may be removed and dried in the sunshine. Following effective drying, the colored spots are identified using a 10% ethanolic KOH reagent and seen under a ultraviolet (UV) chamber (Shimadzu UV-1900i) at 365 nm [16].

Fourier transform infrared (FT-IR) analysis of isolated product

The functional groups of barbaloin are identified using FT-IR (Shimadzu, IR Spirit). FT-IR analysis is essential for the characterization of the moiety.

High-resolution mass spectrometry (HRMS) analysis isolated product

HRMS analysis of isolated moieties necessitates an accurate estimate of their molecular mass and fragmentation pattern, essential for structural confirmation and purity evaluation.

NMR spectrum of isolated product

The NMR spectra of the isolated phytoconstituents (2 mg/mL in D_2O) were acquired using a BRUKER 500 Ultra Shield TM spectrometer in a D_2O solution. The experiments were performed at ambient temperature, ensuring that the solvent (HOD) peak did not interfere with any other peaks. After dissolution, 1 mL of the isolated product solution was transferred to a 5 mm NMR tube. The sample tube was positioned in the magnetic field and allowed to reach thermal equilibrium for 10 min before the experiment began.

Structure elucidation of the moiety

Pure compounds are isolated from *A. vera* extract by column chromatography. Initially, the product will be confirmed using TLC by comparing its R_f value with that of a standard. Subsequently, the integration of FT-IR, HRMS, UV-visible spectroscopy, and NMR (1H, 13C) data facilitated the elucidation of the pure barbaloin structure.

Animals

The present investigation used male Swiss albino rats weighing between 150 and 250 g. The animals were housed in hygienic polypropylene cages and provided with a consistent pellet diet (Hindustan Lever in Kolkata, India) along with unrestricted access to water. Before the commencement of the experiment, the animals were introduced to acclimatize to the laboratory environment, characterized by a temperature of 25±2°C and a 14/10 h light/dark cycle. All operations received approval from TAAB Biostudy Services, Institutional Animal Ethical Committee (No. 1938/P.O./Rc/S/l 7/CPCSEA), Kolkata, India. Anti-cancer study adheres to internationally accepted standards for animal research, following the 3Rs principle.

Acute toxicity test

We used six animals per group (n=6) and split them into five categories. The E.A.F. was administered orally in increasing doses up to 2000 mg/kg body weight [17]. The rats were observed continuously for 2 h for behavioral, neurological, and autonomic profiles, and after 24 h and 72 h for lethality.

In vivo antidiabetic activity

Acute toxicity studies - Fixed dose procedure

Acute oral toxicity investigations were performed following the OECD-420, 2001 criteria. Employing random selection methods, we chose

male albino Wistar rats for the acute toxicity assessment. The animals were originally subjected to an overnight fast, receiving just water. The first control group was given normal saline orally. The ethyl acetate extract of *A. vera* solution was supplied orally at escalating dosages up to 2000 mg/kg of body weight.

Post-treatment, the animals were observed at 15, 30, and 60 min during the first 12 h, followed by monitoring every 4 h thereafter. The animals were monitored for indications of general toxicity and death for 14-day post-treatment.

Induction of experimental diabetes

After acclimatization, male Wistar rats were randomly divided into five groups, each consisting of 6 animals. The first group (group I) received an injection of citrate buffer at a dose of 65 mg/kg of body weight. Rats in groups II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII were fed a high-fat (HF) diet orally for 2 weeks before getting a single intraperitoneal injection of STZ at 45 mg/kg body weight in ice-cold citrate buffer (0.1 M, pH 4.5) to induce diabetes. Our objective was to evaluate a combined HF diet and low-dose STZ model for the induction of type-2 diabetes (T2D) in male rat model. From the literature survey, it was reported that T2D biomarkers would differ significantly between sexes. We have contrasted an HF diet (60 kcal from fat) and STZ injections (30 mg/kg/d for 3 days) with a low-fat (LF) diet (10% kcal from fat). Both sexes gained weight and developed impaired postprandial oral glucose tolerance on the HF+STZ treatment compared to LF. Only male mice on HF+STZ developed fasting hyperglycemia, fasting hyperinsulinemia, and insulin resistance, suggesting that the underlying causes of postprandial hyperglycemia differed between sexes, which confirm the occurrence of T2D. Therefore, in our experimental research, we induced HF diet in oral route for 2 weeks along with a low dose of STZ by intraperitoneal route to induce type 2 diabetes [18,19]. Along with STZ, groups III, IV, and V (the third, fourth, and fifth groups) were given the E.A.F. orally (with purified water) at doses of 50, 250, and 500 mg/kg body weight, starting 3 days after STZ injection and continuing for 28 days. Groups VI, VII, and VIII received isolated barbaloin at doses of 5, 10, and 15 mg/kg body weight, respectively. We have selected the dose of ethyl acetate extract and barbaloin as an isolated compound to treat T2D mellitus as it was reported previously and get knowledge from the literature survey [16]. Groups IX, X, and XI received commercial barbaloin at doses of 5, 10, and 15 mg/kg body weight, respectively. Group XII was given glyburide (a standard medication) at 1 mg/kg/day for 28 days (Table 1). The rats had access to a 5% glucose solution for 48 h after the STZ injection. Rats with blood glucose levels over 250 mg/dl were classified as diabetic and used in subsequent tests.

Oral glucose tolerance test in normal and STZ-induced diabetic rats

All animals received glucose (2 g/kg, orally) 15 min post-dosing. The semi-automated biochemical analyzer (Analytica-705, Recorders and Medicare Systems Pvt. Ltd., Haryana, India) was used to assess glucose levels after the collection of blood samples from the lateral tail vein at 0, 30, 60, 90, and 120-min post-glucose loading (Table 2).

Biochemical estimations

Blood samples were obtained from the retroorbital plexus of the experimental animals after 28 days of observation. We evaluated the serum lipid profiles, including total cholesterol, triglycerides, low-density lipoproteins, and high-density lipoproteins, in addition to the blood glucose level. The evaluated biochemical marker enzymes included serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase levels. A standard kit from Span Diagnostic Limited, Surat, India, was used to spectrophotometrically measure blood insulin levels. A glycosylated hemoglobin kit (AF1040) from Stangen Immunodiagnostics in Hyderabad, India, was used to assess hemoglobin A1C levels. Following the homogenization of the liver from the sacrificed rats with 5 mM shows in Fig. 9 Tris-HCl buffer containing 2 mM ethylenediaminetetraacetic acid at pH 7.4, the liver

Table 1: Effect of *Aloe vera* extract and isolated barbaloin and standard barbaloin on blood glucose levels and serum insulin levels in STZ-induced diabetic rats

Days	Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI	Gr-VII	Gr-VIII	Gr-IX	Gr-X	Gr-XI	Gr-XII
0; BG level (mg/dL)	100.9±0.07	320.9±1.05^{*a}	330.5±1.15^{*b}	325.4±1.17^{*b}	319.7±1.02^{*b}	322.8±1.12^{*b}	333.3±1.15^{*b}	300.2±1.08^{*b}	330.5±1.03^{*b}	331.3±1.03^{*b}	312.2±1.09^{*b}	317.8±1.12^{*b}
0; Serum insulin level (µU/mL)	17.9±0.21	8.87±0.47	8.91±0.33	8.87±0.38	8.92±0.09	8.99±0.56	9.01±0.89	14.91±0.56	8.67±0.45	8.99±0.36	10.99±0.56	8.03±0.71
7; BG level (mg/dL)	101.7±0.05	332.5±1.12^{*a}	232.3±1.15^{*b}	220.3±1.22^{*b}	185.5±1.29^{*b}	312.4±1.11^{*b}	300.2±1.06^{*b}	251.6±0.09^{*b}	310.7±1.05^{*b}	315.5±1.08^{*b}	276.3±1.06^{*b}	212.4±0.08^{*b}
14; BG level (mg/L)	102.3±10.03	340.7±1.25^{*a}	227.5±1.21^{*b}	216.7±1.27	165.7±1.59^{*b}	265.3±1.05^{*b}	266.6±1.07^{*b}	201.3±1.02^{*b}	278.7±1.06^{*b}	276.5±1.01^{*b}	210.5±1.05^{*b}	165.3±0.18^{*b}
28; BG level (mg/dL)	100.9±0.06	357.4±1.15^{*a}	220.6±0.09^{*b}	202.3±1.10^{*b}	122.2±0.95^{*b}	199.5±1.02^{*b}	152.0±1.10^{*b}	124.1±0.03^{*b}	266.1±1.04^{*b}	205.9±1.07^{*b}	152.7±1.03^{*b}	119.5±0.92^{*b}
28; Serum insulin level (µU/mL)	18.7±0.42	8.01±0.05^{*a}	17.3±0.41^{*b}	18.09±0.72^{*b}	18.32±0.33^{*b}	13.9±0.09^{*b}	15.1±0.42^{*b}	18.5±0.45^{*b}	9.82±0.77^{*b}	9.97±0.33^{*b}	12.5±0.45^{*b}	17.99±0.05^{*b}

Values are mean±SD, n=6. STZ (45 mg/kg, b.w) was injected to control and all other drug-treated groups, ^{a}STZ induced diabetic group versus normal group, *p<0.01, ^{b}Treated group versus STZ induced diabetic group, *p<0.01. STZ: Streptozotocin

was subjected to centrifugation at 3000 rpm for 10 min at 4°C. We used commercially available kits and the manufacturer's activity instructions to quantify catalase, malondialdehyde, superoxide dismutase, and glutathione peroxidase in the acquired supernatant (Table 3)

In vivo anticancer activity

Ehrlich ascites carcinoma (EAC) model

EAC cells were injected into the peritoneal cavity of Wistar rats. For the experiment, EAC cells (2×10^6 cells/rat) were intraperitoneally injected into Wistar rats. The rats were randomly divided into six groups (n=6 per group):

1. Normal control (0.9% NaCl, 5 mL/kg)
2. EAC control
3. EAC+*A. vera* extract (50, 250, and 500 mg/kg)
4. EAC+barbaloin (5, 10, and 15 mg/kg)
5. EAC+standard barbaloin (5, 10, and 15 mg/kg)
6. EAC+5-FU (20 mg/kg).

Treatment was administered intraperitoneally daily for 14 days, starting 24 h after EAC cell inoculation.

Assessment of anti-tumor activity

On day 15, blood samples were collected for hematological analysis. Thereafter, the animals were sacrificed, and the following parameters were evaluated (Table 4):

1. Body weight
2. Mean survival time
3. Tumor volume
4. Packed cell volume
5. Viable tumor cell count
6. Non-viable cell count.

Hematological analysis

Blood samples were analyzed for the following parameters (Table 4)

1. Hemoglobin content
2. Red blood cell (RBC) count

Table 2: Effects of *Aloe vera* extract and isolated compounds on oral glucose tolerance test in diabetic rats (Fig. 7)

Groups	Blood glucose level (mmole/L)			
	0 min	30 min	60 min	120 min
Gr-I (Control)	3.91±0.03	7.15±0.35	5.08±0.25	4.89±0.69
Gr-II (STZ);45 mg/Kg body weight	19.55±1.25*[a]	23.36±1.16*[a]	25.55±0.75*[a]	18.67±0.66*[a]
Gr-III (STZ+ethyl acetate fraction of *A.vera;* 50 mg/Kg body weight)	18.11±0.28*[b]	23.19±0.17*[b]	23.36±0.33*[b]	17.59±0.67*[b]
Gr-IV (STZ+ethyl acetate fraction of *A.vera;* 250 mg/Kg body weight)	17.79±0.77*[b]	22.65±0.58*[b]	22.13±1.03*[b]	16.66±0.96*[b]
Gr-V (STZ+ethyl acetate fraction of *A.vera;* 500 mg/Kg body weight)	15.75±0.36*[b]	20.69±0.55*[b]	21.76±0.12*[b]	10.85±0.76*[b]
Gr-VI (STZ+isolated barbaloin; 5 mg/Kg/body weight)	13.39±1.10*[b]	19.76±0.67*[b]	19.99±0.36*[b]	13.76±0.32*[b]
Gr-VII (STZ+Isolated barbaloin; 10 mg/Kg/body weight)	8.96±0.43*[b]	11.36±1.12*[b]	10.65±0.19*[b]	9.75±0.56*[b]
Gr-VIII (STZ+isolated barbaloin; 15 mg/Kg/body weight)	5.59±0.55*[b]	8.67±0.68*[b]	7.17±0.37*[b]	5.35±0.33*[b]
Gr-IX (STZ+Standard barbaloin; 5 mg/Kg/body weight)	17.63±0.36*[b]	21.29±1.22*[b]	22.36±0.63*[b]	16.96±0.79*[b]
Gr-X (STZ+Standard barbaloin; 10 mg/Kg/body weight)	16.23±0.22*[b]	19.56±0.77*[b]	20.36±0.29*[b]	16.66±0.33*[b]
Gr-XI (STZ+Standard barbaloin; 15 mg/Kg/body weight)	12.25±0.42*[b]	11.19±0.19*[b]	8.79±0.73*[b]	8.85±0.22*[b]
Gr-XII (Glyburide; 1 mg/kg body weight/day)	4.12±0.12*[b]	7.55±0.53*[b]	5.51±0.65*[b]	4.99±0.39*[b]

Values are mean±SD, n=6. STZ (45 mg/kg, b.w.) was injected into the control and all other drug-treated groups. [a]STZ-induced diabetic group was compared with a normal group, *p<0.01, [b]Treated group versus STZ-induced diabetic group, *p<0.01

Table 3: Effect of *Aloe vera* extract, isolated and commercial barbaloin on serum lipid profiles, serum biomarkers, lipid peroxidation, and HbA1C level in STZ-induced diabetic rats after treatment

Parameters	Gr-I	Gr-II	Gr-III	Gr-IV	Gr-V	Gr-VI
Total cholesterol (mg/dL)	67.42±1.72	140.2±1.31*[a]	138.8±1.33*[b]	100.5±1.66*[b]	87.6±1.29*[b]	125.5±1.25*[b]
Triglyceride (mg/dL)	66.5±1.02	168.5±0.8*[a]	153.3±1.07*[b]	120.8±0.7*[b]	100.9±0.6*[b]	155.3±1.05*[b]
High-density lipoproteins (mg/dL)	35±0.65	20.4±0.65*[a]	22.1±0.71*[b]	27.6±0.99*[b]	32.3±1.67*[b]	23.8±0.77*[b]
Low-density lipoproteins (mg/dL)	29.5±0.4	76.4±0.6*[a]	59.8±0.5*[b]	44.4±0.9*[b]	31.9±0.8*[b]	63.3±0.5*[b]
Serum glutamic oxaloacetic transaminase (IU/dL)	101.12±1.52	125.5±1.56*[a]	123.13±0.3*[b]	120.29±0.99*[b]	106.65±0.35*[b]	119.79±0.19*[b]
Serum glutamic pyruvic transaminase (IU/dL)	57.22±5.25	93.30±3.31*[a]	92.28±0.88*[b]	89.99±1.09*[b]	72.33±0.63*[b]	87.76±0.66*[b]
Alkaline phosphatase (IU/dL)	126.67±1.37	252.5±1.55*[a]	250.5±0.75*[b]	247.17±0.79*[b]	171.33±0.36*[b]	245.35±1.25*[b]
Malon-dialdehyde (nmol/mg protein)	0.78±0.05	1.78±0.07*[a]	1.76±0.66*[b]	1.69±0.69*[b]	1.66±0.67*[b]	1.63±0.36*[b]
Glutathione peroxidase (μM/mg tissue)	1.8±0.01	0.85±0.05*[a]	0.99±0.12*[b]	1.01±0.01*[b]	1.11±0.11*[b]	1.12±0.19*[b]
Catalase (μM/mg tissue)	2.4±0.03	0.89±0.99*[a]	0.95±0.11*[b]	1.12±0.12*[b]	1.26±0.66*[b]	1.29±0.29*[b]
Superoxide dismutase (U/mg protein)	400.12±2.45	273.33±0.33*[a]	331.33±1.33*[b]	335.35±0.55*[b]	357.71±0.77*[b]	361.16±0.06*[b]
HbA1C (%)	4.45±0.61	5.57±0.47	5.11±0.33	4.43±0.38	4.11±0.11	5.01±0.46
Parameters	**Gr-VII**	**Gr-VIII**	**Gr-IX**	**Gr-X**	**Gr-XI**	**Gr-XII**
Total cholesterol (mg/dL)	90.3±1.12*[b]	65.52±1.55*[b]	138.7±1.56*[b]	126.5±1.99*[b]	96.8±1.58*[b]	63.32±1.87*[b]
Triglyceride (mg/dL)	100.1±1.06*[b]	70.1±1.01*[b]	163.9±0.7*[b]	157.8±1.04*[b]	100.1±1.01*[b]	65.3±1.01*[b]
High-density lipoproteins (mg/dL)	31.3±0.62*[b]	36.6±0.62*[b]	20.9±0.67*[b]	23.6±0.55*[b]	29.7±0.67*[b]	35.5±0.77*[b]
Low-density lipoproteins (mg/dL)	43.3±0.7*[b]	30.3±0.5*[b]	73.8±0.6*[b]	59.7±0.6*[b]	36.9±0.7*[b]	28.9±0.7*[b]
Serum glutamic oxaloacetic transaminase (IU/dL)	110.56±0.16*[b]	99.17±0.77*[b]	117.29±0.33*[b]	111.11±0.11*[b]	105.01±0.01*[b]	99.12±2.5*[b]
Serum glutamic pyruvic transaminase (IU/dL)	61.66±0.76*[b]	55.29±0.99*[b]	83.36±1.67*[b]	72.29±1.19*[b]	61.16±0.66*[b]	56.33±1.13*[b]
Alkaline phosphatase (IU/dL)	190.32±1.19*[b]	128.41±1.14**[b]	242.26±1.36*[b]	176.66±0.67*[b]	133.33±0.33*[b]	130.77±1.87*[b]
Malon-dialdehyde (nmol/mg protein)	1.33±0.33*[b]	1.21±0.12*[b]	1.11±0.11*[b]	0.99±0.19*[b]	0.96±0.33*[b]	0.99±0.09*[b]
Glutathione peroxidase (μM/mg tissue)	1.22±0.02*[b]	1.31±0.13*[b]	1.55±0.25*[b]	1.63±0.36*[b]	1.76±0.33*[b]	1.82±0.02*[b]
Catalase (μM/mg tissue)	1.32±0.23*[b]	1.67±0.77*[b]	1.79±0.29*[b]	1.99±0.39*[b]	2.02±0.22*[b]	1.99±0.09*[b]
Superoxide dismutase (U/mg protein)	369.99±0.09*[b]	372.25±0.37*[b]	375.25±1.75*[b]	381.18±0.68*[b]	389.67±1.36*[b]	388.69±4.39*[b]
HbA1C (%)	4.99±0.69	4.19±0.29	5.35±0.77	5.19±0.87	4.87±0.69	4.03±0.71

Values are mean±SD, n=6. STZ (45 mg/kg., b.w) was injected to control and all other drug-treated groups, [a]STZ-induced diabetic group versus normal group, *p<0.01, [b]treated group versus STZ-induced diabetic group, *p<0.01 (Fig. 6). HbA1C: Hemoglobin A1C

3. White blood cell (WBC) count
4. Differential leukocyte count (monocytes, lymphocytes, neutrophils).

Statistical analysis

All experiments were carried out in triplicate, and data are represented as mean±standard deviation (n=6) [20,21]. All statistical analysis was performed using GraphPad Prism 8.0 software. Analyses were conducted using one-way analysis of variance followed by *post hoc* Dunnett's test, as implemented in version 10 of the Statistical Package for the Social Sciences computer software, to compare multiple groups. $p<0.01$ was considered statistically significant.

RESULTS

Authentication of the plant

This plant was identified and authenticated at the Botanical Survey of India, Shibpur, Howrah, by Dr. S. S. Dash, Scientist E. Plant authentication Ref. No. is BSI-295/I (Misc)/2021-Tech/1725.

Determination of extractive value

After removing the solvent under reduced pressure to get petroleum ether (PEAV, yield 8.5%) and ethanol extract (EEAV, yield 22.5%), respectively.

Determination of barbaloin using TLC

TLC characterization of barbaloin was conducted with a mobile phase composed of ethyl acetate, methanol, and water in a ratio of 100:13.5:10. After drying of the TLC plate was kept in a UV chamber at 365 nm wavelength. After that R_f value was found to be 0.40 and 0.46, respectively, for both isolated and commercial (Standard) barbaloin (Fig. 1).

FT-IR analysis

In FT-IR spectra (Shimadzu, IR Spirit), 3359.39 cm^{-1} indicating the presence of hydroxyl groups (O–H Stretching), 2864.74 cm^{-1} representing the alkane C-H bonds (C–H stretching), and 1600.63 cm^{-1} (C=C stretching in conjugated alkene); 1698.02 cm^{-1} (C=O stretching indicates the presence of Carbonyl groups), 1186.01 cm^{-1} showing ether or ester functionalities, 1572.66 cm^{-1} indicating the presence of aromatic rings (Fig. 2).

HRMS analysis

HRMS (ESI) calculated for $C_{21}H_{22}O_9$ $(M+Na)^+$: 441.11615, found 441.0903 (Fig. 3).

NMR spectral analysis (Fig. 4)

1H NMR: δ 3.03–3.40 (4H, 3.10 (t, J = 10.2 Hz), 3.22–3.24 (t, J = 10.2 Hz), 3.25–3.28 (t, J = 10.2 Hz), 3.35–3.36 (d, J = 10.3, 6.5 Hz)), 3.84–4.00 (3H, 3.91–3.93 (dd, J = 10.3, 2.6 Hz), 3.94–3.95 (d, J = 6.5 Hz), 3.96–3.97 (d, J = 6.5 Hz)), 4.16–4.61 (7H, 4.16 (s), 4.36 (t), 4.41 (d, J = 2.6 Hz), 4.51 (d), 4.54 (t), 4.61 (d), 5.27–5.37 (3H): 5.27 (t), 5.32 (s), 5.37 (s), 6.72–6.89; (2H): 6.78–6.80 (dd), 6.841–6.845 (d), 7.00–7.27; (3H): 7.05–7.09 (dd), 7.171–7.178 (d), 7.23 (t). 13C NMR: δ 44.8 (1C, s), 62.5 (1C, s), 64.7 (1C, s), 71.4–71.5 (2C, 71.4 (s), 71.5 (s)), 76.1 (1C, s), 79.9 (1C, s), 81.4 (1C, s), 114.3 (1C, s), 114.4–114.5 (2C, 114.4 (s), 114.4 (s)), 116.8 (1C, s), 120.7 (1C, s), 124.1 (1C, s), 129.6 (1C, s), 138.0 (1C, s), 140.3 (1C, s), 147.4 (1C, s), 162.0 (1C, s), 162.3 (1C, s), 188.9 (1C, s).

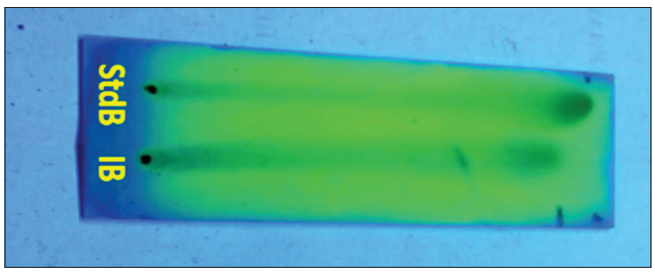

Fig. 1: Thin-layer chromatography chromatogram of commercial and isolated barbaloin

Table 4: Effect of isolated barbaloin and standard barbaloin on tumor parameters in EAC-bearing Wistar rats and several biological parameters

Treatment	Dose (mg/kg)	Body wt (g)	MST (Days)	Tumor volume (mL)	Packed cell volume (mL)	Viable tumor cell count ($\times10^3$cells/mL)	Non-viable cell count ($\times10^3$ cells/mL)	Hb (g/dL)	RBC (cells/ mL×10≥)	WBC (cells/ mL×10≥)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)
EAC control	-	26.20±0.25	18.35±1.55	4.99±0.22	2.44±0.33	11.33±0.33	0.91±0.55	10.09±0.33	2.25±0.45	20.15±0.45	1.09±0.01	26.64±0.36	62.63±0.76
EAC+EA *A. vera* Ext.	50 mg/Kg	26.06±0.33	18.65±0.55	3.35±0.35	1.98±0.22	3.05±0.29	0.97±0.55	11.09±0.25	2.79±0.19	18.75±0.45	1.01±0.02	49.87±0.77	44.44±0.45
EAC+EA *A. vera* Ext.	250 mg/Kg	26.02±0.43	18.88±0.36	3.22±0.66	1.79±0.19	2.65±0.12	0.99±0.39	11.56±0.23	3.33±0.22	17.65±0.65	1.11±0.15	52.39±0.33	52.25±0.25
EAC+*A. vera* Ext.	500 mg/Kg	25.98±1.22	19.75±0.37	2.85±0.33	1.65±0.26	2.22±0.19	1.01±0.32	11.66±0.25	3.76±0.99	15.55±0.35	1.25±0.55	55.55±0.75	60.25±0.75
Standard barbaloin	5	22.75±0.56	23.05±1.01	1.39±0.22	0.69±0.33	1.39±0.55	1.17±0.65	11.90±0.55	4.78±0.76	10.55±0.55	1.52±0.55	60.55±0.75	39.45±0.16
Standard Barbaloin	10	22.25±0.35	25.39±0.19	1.33±0.19	0.55±0.25	1.26±0.25	1.25±0.39	12.25±0.52	4.97±0.99	10.01±0.12	1.69±0.11	68.85±0.15	36.66±0.33
Standard barbaloin	15	21.47±0.33	28.75±0.25	0.73±0.11	0.37±0.66	0.87±0.39	1.28±0.56	13.69±0.66	5.55±0.25	9.33±0.33	1.76±0.77	70.15±0.55	33.33±0.33
Isolated barbaloin	5	24.33±0.47	20.22±0.22	1.76±0.16	0.88±0.21	2.32±0.54	0.99±0.12	10.70±0.65	4.14±0.12	13.20±0.25	1.33±0.33	55.40±0.85	42.20±0.12
Isolated Barbaloin	10	23.65±0.55	21.05±0.31	1.47±0.19	0.72±0.15	2.02±0.33	1.10±0.19	11.05±0.65	4.65±0.55	12.01±0.15	1.35±0.55	57.77±0.17	40.15±0.19
Isolated Barbaloin	15	23.22±0.54	21.75±0.27	1.39±0.22	0.57±0.39	1.97±0.56	1.22±0.33	12.22±0.25	4.97±0.75	11.99±0.99	1.55±0.15	63.33±0.33	39.99±0.19
5-FU	20	21.47±0.36	30.18±0.22	1.49±0.19	0.20±0.12	0.76±0.51	1.31±0.26	13.77±0.75	5.25±0.11	8.20±0.11	1.80±0.16	72.18±0.22	28.08±0.43

Values are mean±SD, n=6. 5-FU (20 mg/kg, b.w) was injected to control group and all other drug-treated groups, *p<0.01. 5-FU: 5-Fluorouracil, RBC: red blood cell, WBC: White blood cell, EAC: Ehrlich ascites carcinoma, Hb: Hemoglobin

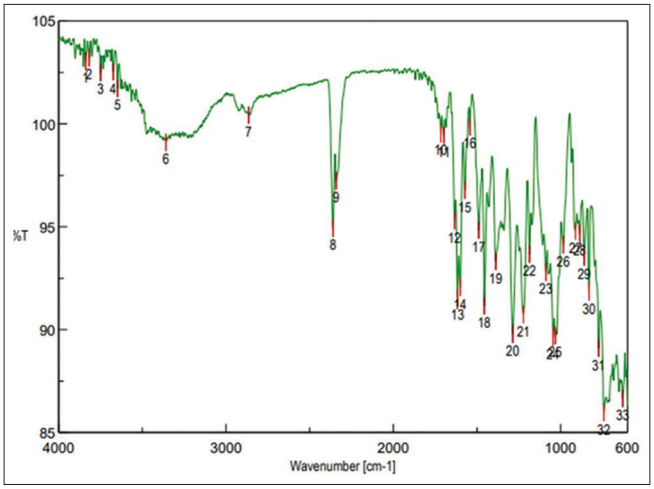

Fig. 2: Fourier transform infrared spectra of isolated barbaloin

Structure elucidation

Yellowish-brown color; 1H NMR: δ 3.03–3.40 (4H, 3.10 (t, J = 10.2 Hz), 3.22–3.24 (t, J = 10.2 Hz), 3.25–3.28 (t, J = 10.2 Hz), 3.35–3.36 (d, J = 10.3, 6.5 Hz)), 3.84–4.00 (3H, 3.91–3.93 (dd, J = 10.3, 2.6 Hz), 3.94–3.95 (d, J = 6.5 Hz), 3.96–3.97 (d, J = 6.5 Hz)), 4.16–4.61 (7H, 4.16 (s), 4.36 (t), 4.41 (d, J = 2.6 Hz), 4.51 (d), 4.54 (t), 4.61 (d), 5.27–5.37 (3H): 5.27 (t), 5.32 (s), 5.37 (s), 6.72–6.89; (2H): 6.78–6.80 (dd), 6.841–6.845 (d), 7.00–7.27; (3H): 7.05–7.09 (dd), 7.171–7.178 (d), 7.23 (t). 13C NMR: δ 44.8 (1C, s), 62.5 (1C, s), 64.7 (1C, s), 71.4–71.5 (2C, 71.4 (s), 71.5 (s)), 76.1 (1C, s), 79.9 (1C, s), 81.4 (1C, s), 114.3 (1C, s), 114.4–114.5 (2C, 114.4 (s), 114.4 (s)), 116.8 (1C, s), 120.7 (1C, s), 124.1 (1C, s), 129.6 (1C, s), 138.0 (1C, s), 140.3 (1C, s), 147.4 (1C, s), 162.0 (1C, s), 162.3 (1C, s), 188.9 (1C, s). In FT-IR spectra, 3359.39 cm^{-1} indicating the presence of hydroxyl groups (O–H Stretching), 2864.74 cm^{-1} representing the alkane C-H bonds (C–H stretching), and 1600.63 cm^{-1} (C=C stretching in conjugated alkene); 1698.02 cm^{-1} (C=O stretching indicates the presence of carbonyl groups), 1186.01 cm^{-1} showing ether or ester functionalities, 1572.66 cm^{-1} indicating the presence of aromatic rings. HRMS (ESI) calculated for C21H22O9 $(M+Na)^+$: 441.11615, found

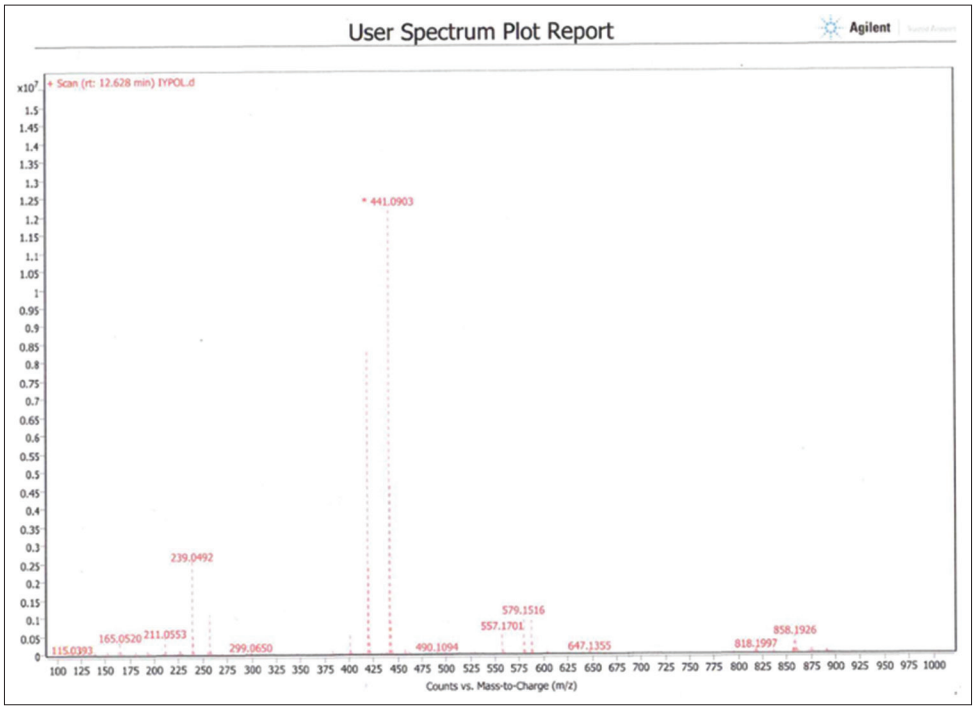

Fig. 3: High-resolution mass spectrometry spectra

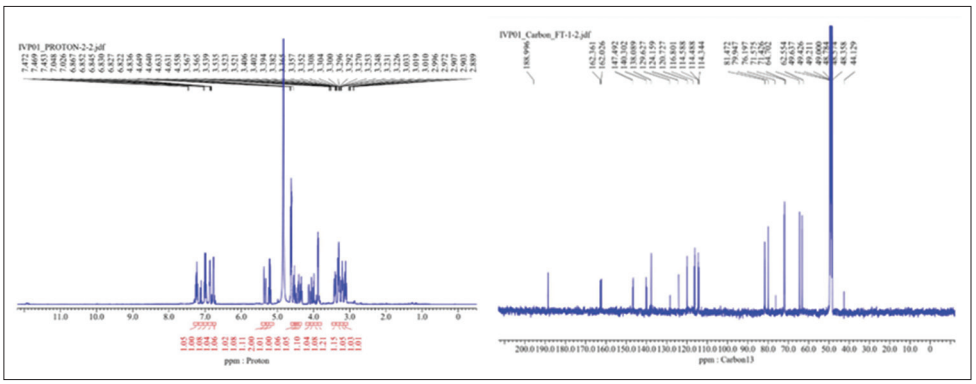

Fig. 4: 1H and 13C NMR of barbaloin

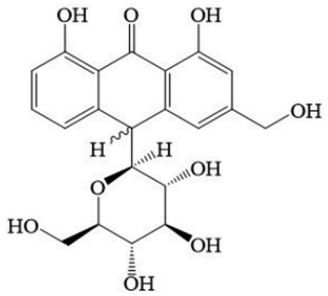

Fig. 5: Structure of isolated compound (barbaloin)

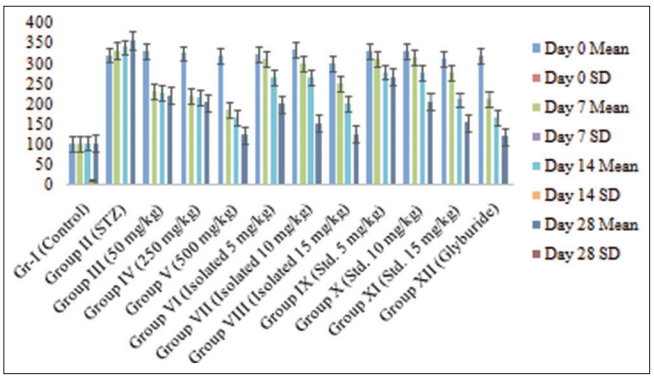

Fig. 6: Effects of blood glucose level

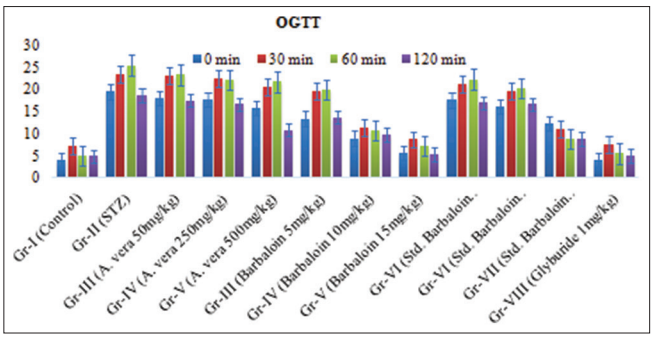

Fig. 7: Determination of oral glucose tolerance test

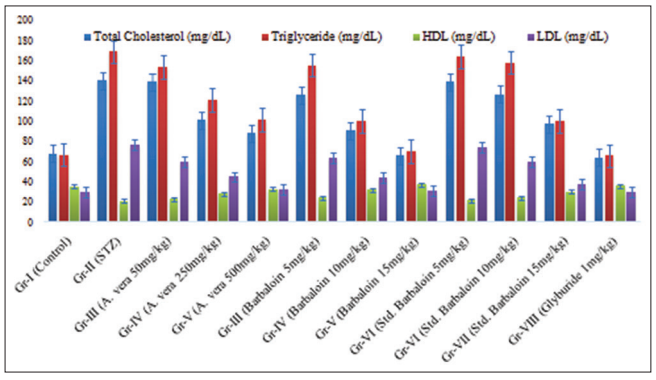

Fig. 8: Determination of serum lipid profile

441.0903. From the above data, the isolated compound was identified as barbaloin. Molecular structure shows in Fig. 5.

***In vivo* antidiabetic activity**

For the Antidiabetic activity in the animal model, we have followed the standard OECD guidelines. The results are as follows for all tests.

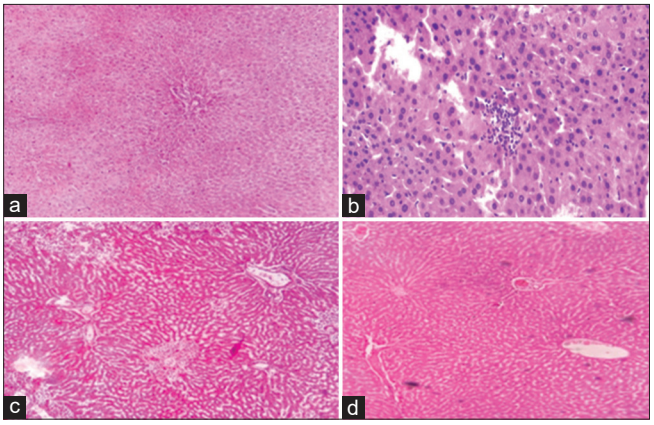

Fig. 9: Histological analysis of the liver (a) Group-V (b) Isolated barbaloin (c) Standard barbaloin

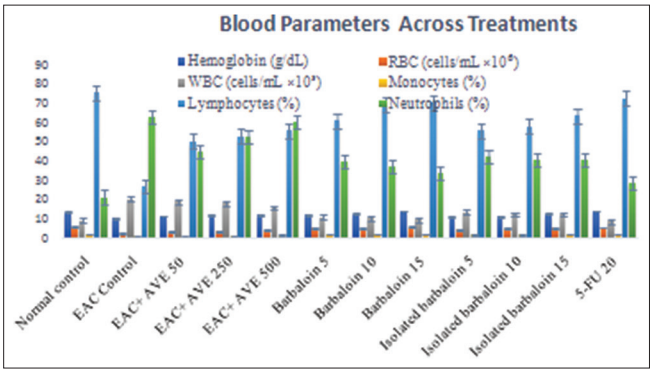

Fig. 10: Determination of several blood parameters

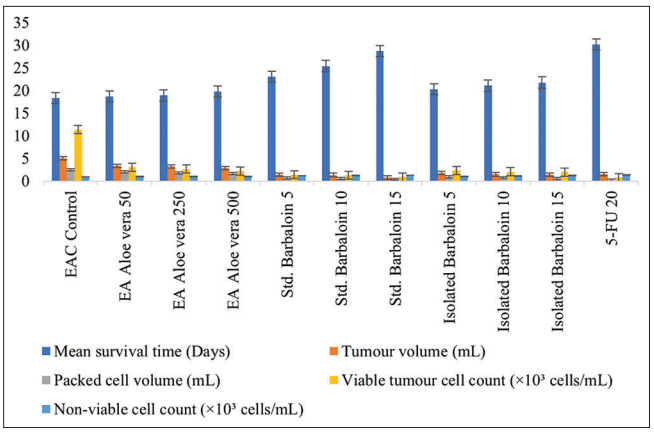

Fig. 11: Determination of several tumor parameters

***In vivo* anti-tumor activity**

Treatment with isolated barbaloin and standard barbaloin demonstrated significant anti-tumor effects in the EAC model in Wistar rats. Both compounds resulted in a dose-dependent decrease in tumor volume, packed cell volume, and viable tumor cell count compared to the EAC control group. At the highest dose (15 mg/kg), standard barbaloin reduced tumor volume by 85.4% and viable tumor cell count by 92.3%, isolated barbaloin reduced these parameters by 72.1% and 82.6%, respectively (Table 4 and Fig. 11).

Moreover, treatment with isolated barbaloin and standard barbaloin significantly produces a higher mean survival time of tumor-bearing rats. The mean survival time increased from 18.35±1.55 days in the

EAC control group to 25.39±0.19 and 21.75±0.27 days in the groups treated with 15 mg/kg of isolated barbaloin and standard barbaloin, respectively.

Hematological parameters

EAC-bearing rats showed significant alterations in hematological parameters, including decreased hemoglobin value and RBC count, and increased WBC count. Treatment with isolated barbaloin and standard barbaloin resulted in a dose-dependent improvement in these parameters. At the highest dose (15 mg/kg), barbaloin increased hemoglobin content from 10.09±0.33 g/dL in the EAC control group to 13.69±0.66 g/dL, standard barbaloin increased it to 12.22±0.25 g/dL (Table 4, Fig. 10). Similarly, both compounds significantly reduced the elevated WBC count and normalized the differential leukocyte count, bringing the values closer to those of normal control rats. These results suggest that isolated barbaloin and standard barbaloin treatment can help to alleviate the hematological abnormalities associated with tumor progression.

CONCLUSION

The work entails the separation of the bioactive secondary metabolite, barbaloin, from the E.A.F. of *A. vera*. This work illustrates the antihyperglycemic efficacy of the E.A.F. and the separated chemicals in STZ-induced diabetic rats. The restoration of metabolic and antioxidant state may explain the protective benefits. It is noteworthy that barbaloin has shown considerable anti-hyperglycemic efficacy. In the case of commercial barbaloin, it has slightly better anti-hyperglycemic efficacy. So that the above animal study data, we can easily conclude that our isolated barbaloin is pure, non-toxic in nature and has antidiabetic potential at very low dose.

This research concludes that both isolated barbaloin and standard barbaloin have substantial anti-cancer properties. Both substances exhibit cytotoxicity, induce apoptosis, promote G2/M phase cell cycle arrest, and regulate the expression of critical proteins associated with cell cycle regulation and apoptosis. These results provide a compelling justification for the further exploration of isolated barbaloin and standard barbaloin, both separately, as prospective anti-cancer medicines. As per the previous reported study, it was established that *A. vera* lowered blood glucose and lipid levels and significantly lowered visceral fat by altering gene expression levels in glucose and lipid metabolism [22]. By increasing insulin sensitivity and decreasing body fat, *A. vera* supplements triggered the enzyme AMP-activated muscle protein kinase, which metabolizes glucose and fat [23]. Future research should focus on clarifying the intricate molecular underpinnings of their synergistic effects, improving combination ratios, and exploring possible interactions with current cancer medicines. Furthermore, pharmacokinetic and toxicological investigations of the combination will be essential to evaluate its potential for clinical use. Although the findings are encouraging, more preclinical and clinical investigations are required before these compounds can be deemed suitable for therapeutic use in cancer patients. *Aloe vera* contains complex mixtures which can be used historically in various pharmacological activities among them as skin ulceration management due to diabetes can also be treated [24]. Higher in the number of diabetic patients, costly medical treatments with unsatisfactory response and less confidence in people in recent days signify the still unsatisfactory nature of the modern medicinal system. These factors are the vital reasons for the continuous faith of people in the traditional medicinal systems. All the important traditional medicinal systems throughout Indian supply high volume evidence for the effectiveness of traditional medicine. It can be justified that important constituents of various traditional medicinal systems can provide a development of more effective drugs in the modern medicinal system [25].

Future aspects

In this *in vivo* investigation on anticancer and antidiabetic effects, the substances isolated barbaloin and standard barbaloin demonstrate considerable efficacy across many anticancer and antidiabetic parameters. These two compounds and their dosage fulfil significant features. During this work, the researchers encountered challenges in pharmacokinetics and pharmacodynamics owing to the inability to identify a specific cell signaling mechanism. Future scholars must concentrate on these aspects. During the studies, no possible bias or restrictions were identified; nonetheless, additional investigation is required about the mechanism of our drug in relation to the signaling pathways implicated in cancer growth. Owing to the absence of this facility at our institution, we are unable to execute this component; nonetheless, we want to focus on certain routes using this substance in the subsequent phase of the project.

CONFLICT OF INTEREST

The authors had no any potential conflict of interest.

FUNDING

Nil.

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