Section A-Research paper
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PHYTOCHEMISTRY AND PHARMACOLOGICAL INVESTIGATION ON DIEXTRACT OF ALBIZIA LEBBECK AND COMBRETUM INDICUM ON DIABECTIC WOUND HEALING ACTIVITY ON EXPERIMENTAL ANIMALS

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ABSTRACT

The medicinal plant Albizia lebback & comberetum indicum is a prospective source of a range of activities, and may be useful as preventative agents in the pathogenesis of numerous ailments, according to the findings of the current study. However, the strength of the available information is insufficient to propose a prudent course of action. The active ingredients that are responsible for the compound's antioxidant action must also be identified, as well as any compound synergism that may exist. This requires additional photochemical research. According to blood biochemical markers, Albizia lebback & Comberetum indicum 100and 200mg/kg B.W have anti-diabetic potential in STZinduced diabetes. Assessment blood glucose level, total cholesterol, triglyceride, HDL-C, total protein, SGPT, SGOT and wound healing activity of Albizia lebback & Comberetum indicum extract

1. INTRODUCTION

1.1 Wound:

When the skin's epithelia line integrity is compromised or live tissue loses or breaks its cellular, anatomical, or functional continuity, the condition is referred to as a wound. The Wound Healing Society defines wounds as unintentional cracks or openings in the skin that interfere with its normal structure and function. In either case, they result in the discontinuity of the epithelium and the damage of causal connective tissue (Ramzie et al., 1994; Strodtbeck, 2001).

1.2. Diabetes:

The first description of diabetes, which was widespread in almost all ancient cultures, including those in Asia, Europe, and America, may be found in the 12 BC Ebers Papyrus from Egypt. Chronic hyperglycemia, issues with carbohydrate metabolism, and issues with protein metabolism are all symptoms of diabetes mellitus. Diabetes arises when the pancreas

cannot make enough insulin or when the body cannot use the insulin that is hormone that regulates blood sugar levels

, hyperglycemia is a typical sign of uncontrolled diabetes. Uncontrolled diabetes has the potential to gravely impair several bodily functions over time, including blood vessels and neurons (WHO, 2006). Chronic hyperglycemic diabetes has been produced. Since insulin is a crucial

related to chronic damage, dysfunction, and letdown of numerous tissues, including the kidneys, bloodvessels, kidneys, eyes, nerves, and kidneys, (according to Jadav and Watermelon 2012).

2. Material and method:

2.1 Collection of plant: Collection of Combretum indicum flowers and Albiza lebbeck pods from C.C.S. Botanical Garden in Meerut, Uttar Pradesh, on December 22.

2.2 Authentication: Combretum indicum flowers and pods from Albiza lebbeck were authenticated by CSIR, Delhi and verified by Dr. Sunita Garg. The number of the authentication certificate is NIScPR/RHMD/Consult/2023/4391-92-1 & NIScPR/RHMD/Consult/2023/4391-92-2.

2.3 Extraction process:

Soxhlet Extraction

Procedure

The albiza lebbeck pods and the combretum indicum flowers were collected, dried, powdered, combined, and fed into the main chamber of the Soxhlet extractor in a thimble made of thick filter paper. The Soxhlet extractor was housed in a flask filled with extraction solvent. The Soxhlet was then equipped with a condenser.

The solvent was heated through reflux. The solvent vapour rises up a distillation arm and pours into the chamber containing the thimble of solid. The condenser causes any solvent vapour to cool and trickle back down into the chamber containing the hard material. Progressively warm solvent is poured into the chamber holding the solid material. Then, the heated solvent was used to partially dissolve the selected chemical.

When the Soxhlet chamber was nearly full, a side arm syphon automatically emptied it, and the solvent then automatically ran back down to the distillation flask. You might let this cycle go on for several hrs or even days. A fraction of the nonvolatile component dissolves in the solvent after each cycle. The required

component is concentrated in the distillation flask after a number of cycles. When the solvent is gone, the insoluble portion of the recovered substance is maintained in the thimble and thrown away.

2.4 Analysis of the Qualitative Photochemical:

Carbohydrate test:

The extract was filtered after a little quantity of it was mixed in 5 ml of distilled water. The remains was examined to look for different photochemical components in the sample.(JM jagi 2006)

Molisch's test

2-3 ml of filtrate was dissolve with a few droplets of Molisch's reagent, and the test tube walls were then coated with strong sulfuric acid. A violet coloured ring emerges when two liquids come together, indicating the presence of carbohydrates.

Fehling's test

1ml of Fehling's- B (potassium tartrate and sodium hydroxide in distilled water) mixture was combined with 1ml of Fehling's-A (copper sulphate in distilled water), which was then heated for a minute. This was cooked gradually after adding 1ml of the filter. Reducing sugars are present when a brick-red precipitate forms.

Benedict's test

A little amount of filtrate was combined with an equivalent amount of Benedict's reagent, an alkaline solution containing cupric citrate complex, and heated for five minutes in a pot of boiling water. The presence of reducing sugars is implied by the formation of a reddish brown precipitate.

Test for Alkaloids

Little extract combined with a few millilitres of diluted hydrochloric acid. Filtered and well-shaken the collected filtrate was used for the assays that follow.

Dragendorff's test

1. Prepare Dragendorff's reagent by mixing 10 g of potassium bismuth iodide and 10 g of potassium iodide in 100 mL of acetic acid.

2. Add a small amount of the sample to a test tube.

3. Add a few drops of Dragendorff's reagent to the test tube.

4. Observe the test tube for the formation of a precipitate.

If a precipitate forms, it is an indication that the sample contains alkaloids. The color of the precipitate can vary depending on the type of alkaloid present. For example, morphine

produces a red precipitate, while quinine produces a yellow precipitate

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The Mayer test

Here are the steps on how to perform the Mayer test:

1. Prepare Mayer's reagent by mixing 1.36 g of mercuric chloride and 5.00 g of potassium iodide in 100 mL of water.
2. Add a small amount of the sample to a test tube.
3. Add a few drops of Mayer's reagent to the test tube.
4. Observe the test tube for the formation of a precipitate.

If a precipitate forms, it is an indication that the sample contains alkaloids. The color of the precipitate can vary depending on the type of alkaloid present. For example, morphine produces a cream-colored precipitate, while quinine produces a yellow precipitate.

The Wagner test

Here are the steps on how to perform the Wagner test:

1. Prepare Wagner's reagent by mixing 1.00 g of bismuth chloride and 1.00 g of potassium iodide in 100 mL of hydrochloric acid.
2. Add a small amount of the sample to a test tube.
3. Add a few drops of Wagner's reagent to the test tube.
4. Observe the test tube for the formation of a precipitate.

If a precipitate forms, it is an indication that the sample contains alkaloids. The color of the precipitate can vary depending on the type of alkaloid present. For example, morphine produces a reddish-brown precipitate, while quinine produces a yellow precipitate.

Steroids and terpenoids detection test:

50 mg of the abstract were combined with 2 ml of chloroform and 2 ml of strong sulfuric acid, and then everything was thoroughly shaken. then saw how the layers of acid and chloroform were coloured. The presence of steroids is indicated by the chloroform layer's appearance as a red colour and the acid layer's appearance as a greenish yellow fluorescence..

Liebermann -Burchard Test

A test tube containing 50 mg of the extract was filled with 2 ml of acetic anhydride, 2 ml of chloroform, and heated to boiling before cooling. The test tube was then filled with 1 ml of concentrated sulfuric acid, and the production of colour at the junction was watched for

.

The presence of steroidal terpenoids is indicated by the development of red, pink, or violet colour at the liquid-liquid interface.

Glycoside test

Legal's test

To 1ml of extract, 1ml of pyridine and 1ml of sodiumnitroprusside were dissolved. The presence of glycosides is indicated by a pink to red colour.

Test Keller-Killiani

Two milliliters of the extract were treated with glacial aceticacid, a trace amount of ferric chloride, and two to three drops of strong sulfuric acid. When two liquids converge, a reddish brown color develops, signaling the presence of cardiac glycosides.

The tannin and phenolic compounds identification

Froth test:

Sodium bicarbonate solution was added to a test sample volume of 5ml. The mixture should be held for three minutes after vigorous shaking. Saponins are present when a froth formation resembling a honeycomb forms.

Test for Flavonoids

Alkaline reagent test

Here are the steps on how to perform the alkaline reagent test:

Ferric chloride testing: To 2ml of distilled water, 1ml of the abstract's alcoholic solution was added. A few drops of 10% ferric chloride were then added. Phenols are present when blue or green colour develops.

Test for lead acetate

5ml of an aqueous extract has a few drops of lead acetate dissolve to it. The presence of tannins is indicated by the precipitation of yellow or red colors.

Test for Saponins

Foam Test:

A test sample of one milliliter was diluted with twenty milliliters of distilled water and shaken in a graduated cylinder for three minutes. The presence of saponins is indicated by a 1 cm foam after 10 minutes.

1. Prepare alkaline reagent by mixing 10g of sodium hydroxide in 100 ml of H_2O
2. Add a small amount of the sample to a test tube.
3. Add a few drops of alkaline reagent to the test tube.
4. Observe the test tube for the formation of a precipitate.

If a precipitate forms, it is an indication that the sample contains flavonoids. The color of the precipitate can vary depending on the type of flavonoid present. For example, quercetin produces a yellow precipitate, while kaempferol produces a green precipitate.

Magnesium hydrochloride reduction test, or the Shinodas test:

Before adding a few drops of strong HCl, a tiny piece of magnesium ribbon was employed to treat the extract's alcoholic solution. The appearance of crimson red, or occasionally green to blue tint, suggests the presence of flavonoid.

Test for Proteins and Amino Acids
Screening for biuret

3To a 4% sodium hydroxide solution, a tiny amount of a 1% copper sulphate solution and 3ml of the test solution were added. A violet hue appears when proteins are present.

2.5.2 Estimation of Total Cholesterol (TC):

Estimation of Total Cholesterol (TC) 3 the serum's total cholesterol was determined using CHOD/PAP methods

The ninhydrin test

Three milliliters of the test solution and three drops of a 5% Ninhydrin solution were cooked for ten minutes in a pot of boiling water. A purple or bluish indicates the presence of free amino acids.

2.5 Anti-diabetic screening
2.5.1 Estimate glucose tolerance test:

Estimate from an oral glucose tolerance test Oxygen and glucose interact in the occurrence of glucose-oxidase to produce gluconicacid and hydrogen peroxide. When hydrogen peroxide oxidises the dyes, a process mediated through peroxidase, the pigments turn blue. However, it was found that the glucometer was useful for diagnostics in terms of test correctness, anywhere a droplet of plasma is adequate to obtain the findings and it is simple to access at room temperature or higher than 95° F. biochemical indicators.

(Tietz, 1995). In adding to presence a vital constituent of cell membranes and lipoprotein, cholesterol is used as a starting material for the production of steroid hormones and bileacids.

3. Results and discussion:

3.1 Photochemical screening of extracts

Table 3.1: Presents of results in several parts of Albizia layback.

Phytochemical	Ethyl Acetate	Methanol	Water
Alkaloids	-	+	-
Glycosides	+	-	-
Tannin	+	+	+
Saponin	+	+	+
Steroid	-	-	-
Flavanoids	+	+	+
Carbohydrates	+	+	+
Amino Acid	+	-	-
Proteins	+	-	-

Presents of results in several parts of comberetum indicum.

3.2 Results of Anti-diabetic activity

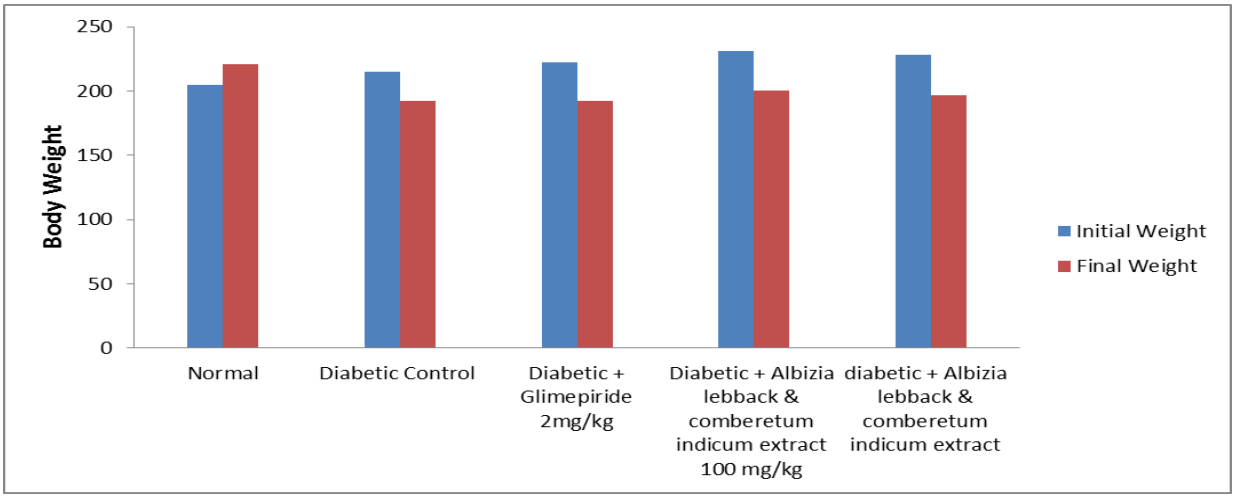

Figure3.1:Mean Body Weight Change

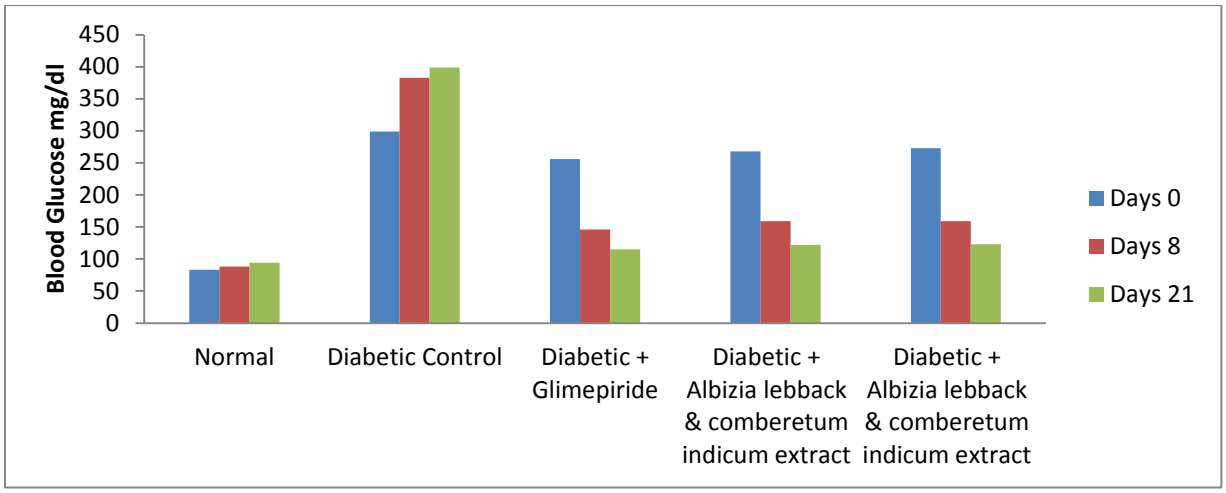

Figure 3.2: Antidiabetic activity of *Albizia lebback & comberetum indicum extract* on blood glucose level in STZ-induced diabetic rats

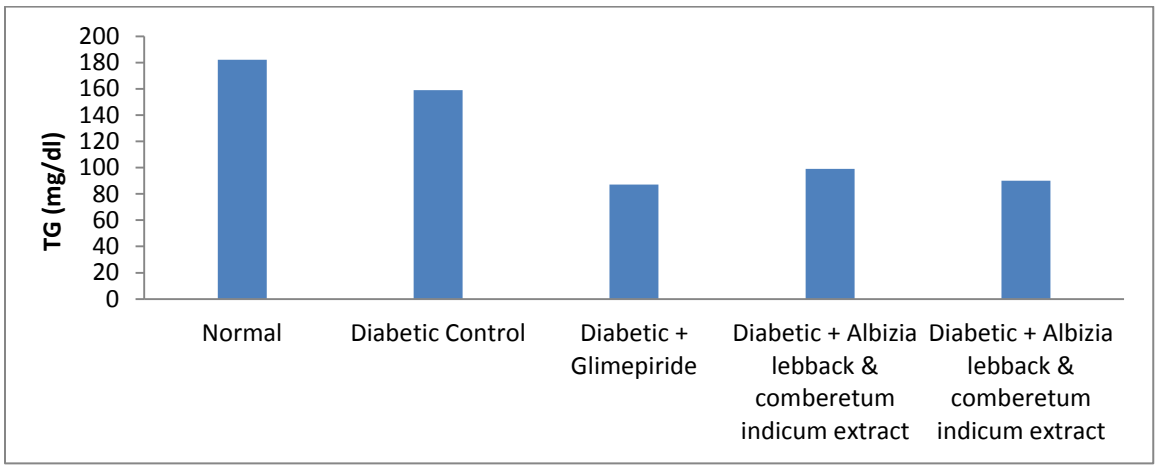

Figure 3.4: Effect of *Albizia lebback & comberetum indicum extract* on triglyceride level in STZ-induceddiabeticrats

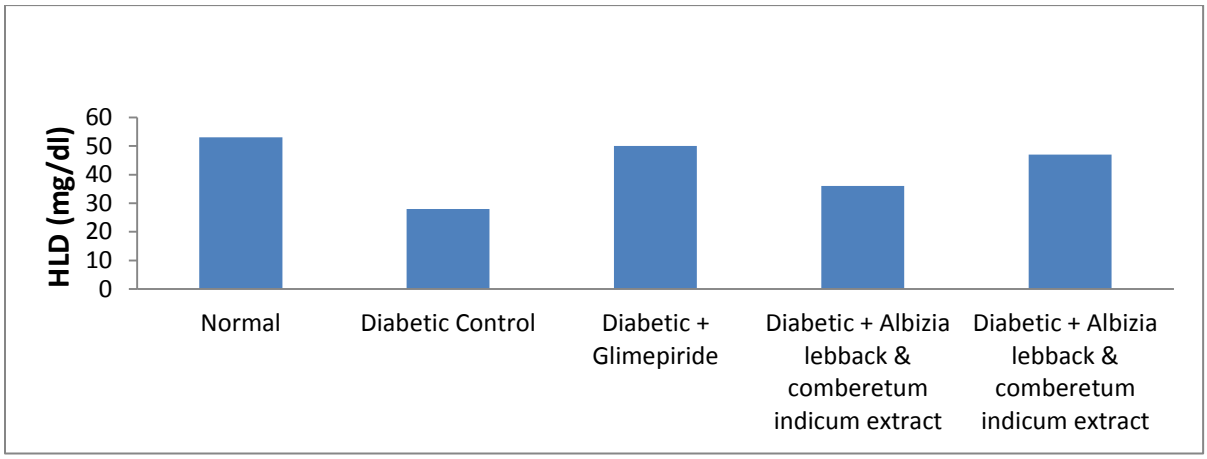

Figure 3.5: Effect of *Albizia lebback & comberetum indicum extract* on HDL in STZ-induced diabeticrats

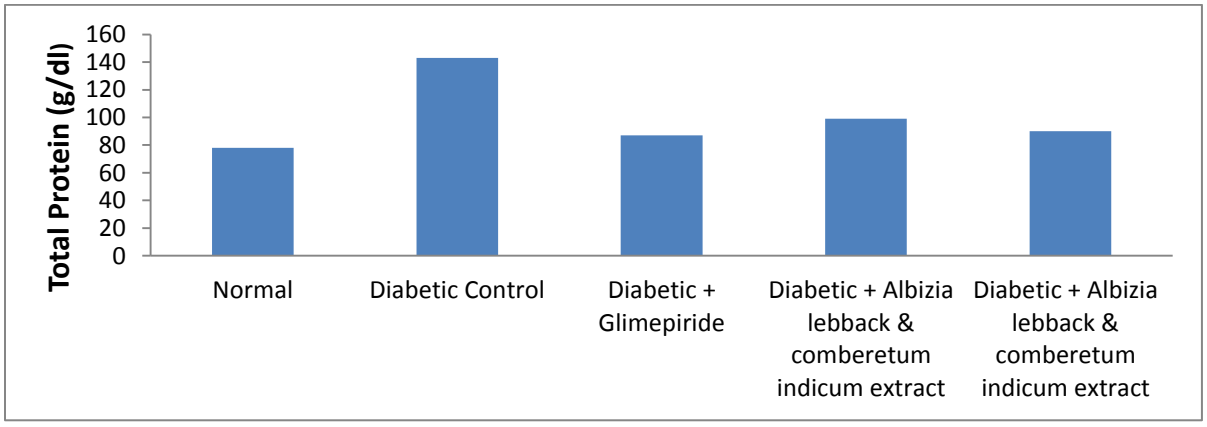

Figure3.5:Effect of *Albizia lebback & comberetum indicum extract* on TP in STZ induced diabetic-rats

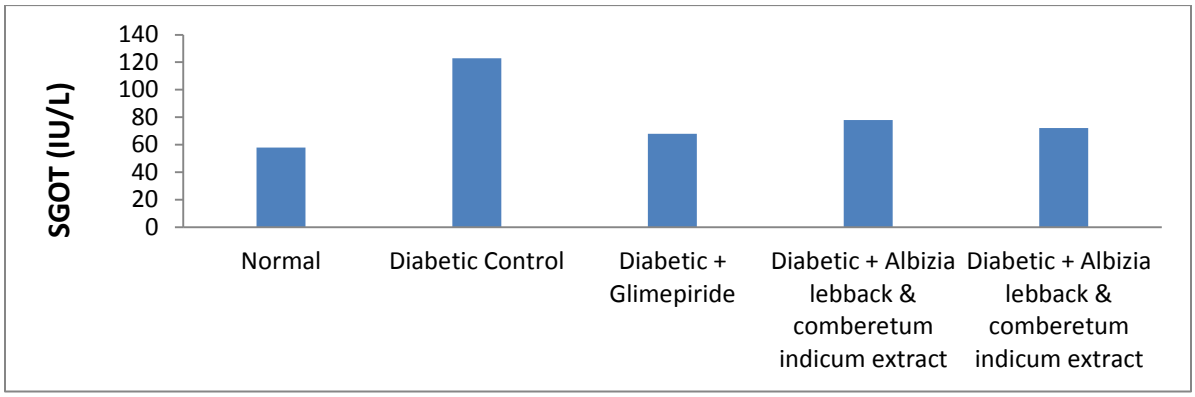

Figure3.6: Effect of *Albizia lebback & comberetum indicum extract* on SGOT in STZ-induced diabetic-rats

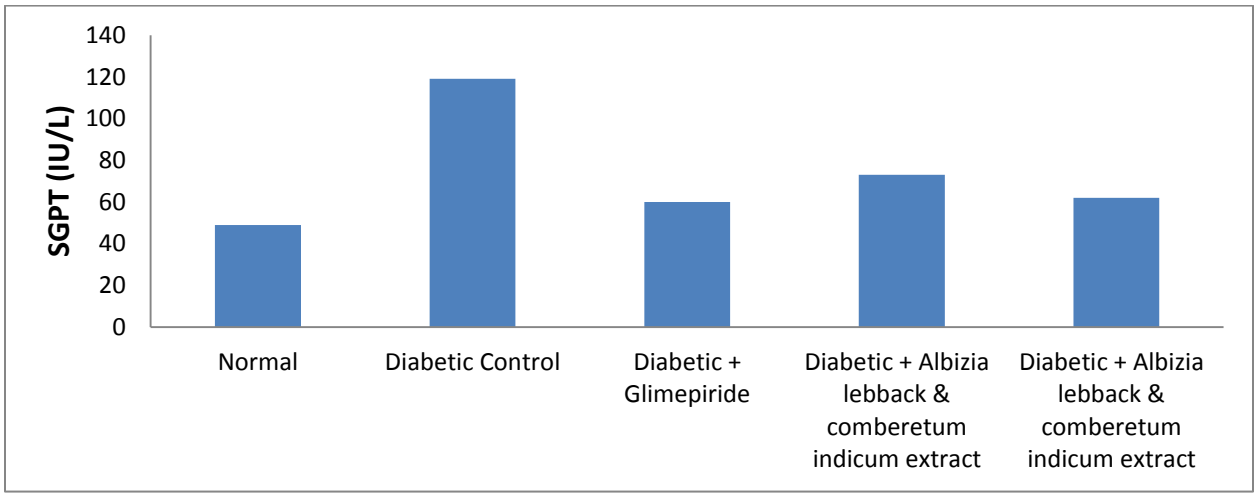

Figure 379: Effect of *Albizia lebback & comberetum indicum extract* on SGPT in STZ-induced diabeticrats

3.3. Results of wound healing activity

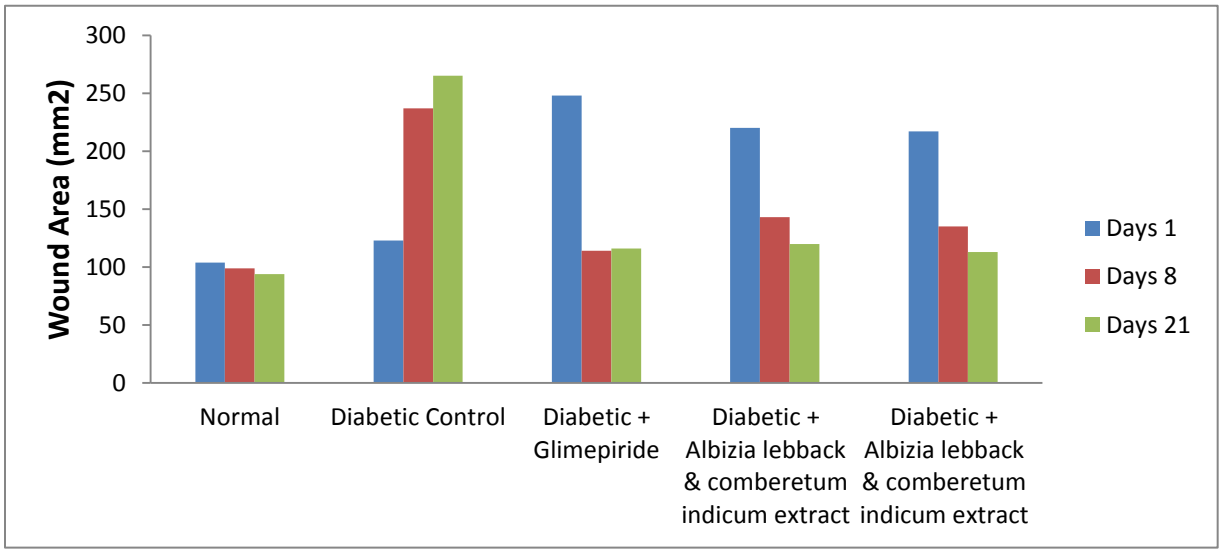

Figure 3.7: Effect of *Albizia lebback & comberetum indicum extract* treatment on Excision Wound [Wound Area (mm2)]

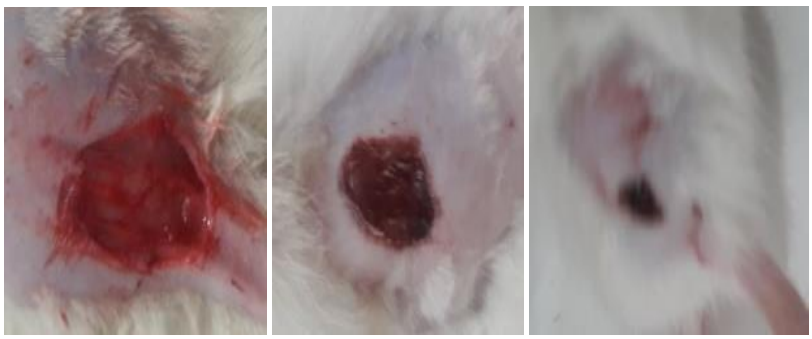

Figure3.8: the wound diameter on the different days A)1 day, B) 8day and C)21Day

3.4 Discussion:

Intraperitoneal injection with STZ-efficiently induced-diabetes mellitus in normal-rats, as shown by blood glucose levels and body mass loss in comparison to normal rats. Albizia lebback and Comberetum indicum have an anti-diabetic effect, according to the research, as evidenced by the fact that they reduced plasma glucose levels in STZinduced diabetic-rats. Throughout the course of the examination, blood

glucose levels fell in all therapy groups in our study. All therapy groups experienced a steady decline in plasma glucose-levels completed the sequence of the study. At the conclusion of the experiment, albizia lebback & comberetum indicum 100 and 200 mg/kgp.o. and glimepiride 2 mg/kgp.o. Were all given (115.00 ± 8.50; 122.00 ± 8.00 and 123.00 ± 7.50) Blood glucose levels in the treated group considerably decreased, which was associated with polyphagia and weight loss brought on by an excessive breakdown of tissue proteins. Dehydration and the breakdown of proteins and fats in diabetic rats may be the cause of their decreased body weight (Wang et al., 2015).

The results demonstrated that extract treatment considerably lowered the serum cholesterol levels in diabetic rats. Additionally, total cholesterol in both the Albizia lebback & comberetum indicum treated group and the untreated group at 100 and 200 mg/kg/p.o. decreased significantly ($p< 0.05$) (141.00± 8.00; 128.00± 7.00). When compared to the control group (215.00± 6.00), totalcholesterol suggestively lowered ($p<0.05$) in the 2 mg /kg Glimepiride-treated group (113.00 ±7.00). Additionally, at 100and 200mg/kg/p.o. (99.00 ±9.00; 90.00± 8.00), the triglyceride levels in the Albizia lebback & comberetum indicum treated group decreased significantly ($p <0.05$). When compared to the control group (159.12±9.00), triglycerides were significantly reduced ($p< 0.05$) in the 2 mg/kg Glimepiride-treated group (87.00±8.00).

The outcomes demonstrated that extract therapy dramatically lowered serum cholesterol levels in diabetic rats. Additionally, total cholesterol significantly decreased in the Albizia lebback & comberetum indicum treated group at 100 and 200 mg/kg/p.o. (141.00± 8.00; 128.00± 7.00) ($p< 0.05$). Additionally, the treated group's total cholesterol level dramatically decreased ($p< 0.05$). When compared to the control group, total cholesterol significantly lowered ($p< 0.05$) in the 2 mg/kg Glimepiride-treated group (215.00± 6.00) (113.00± 7.00). Additionally, at 100 and 200 mg/kg/p.o. (99.00± 9.00; 90.00 ±8.00), the triglyceride levels in the Albizia lebback & comberetum indicum treated group were considerably lower ($p< 0.05$). In the 2 mg/kg Glimepiride-treated group (87.00± 8.00), compared to the control group (159.12± 9.00), triglycerides significantly lowered ($p< 0.05$). In addition, total protein (TP) significantly decreased in the diabetes

control group ($p < 0.001$) and increased in the Albizia lebback & comberetum indicum 100 mg/kg (99.05± 7.00) and Albizia lebback & comberetum indicum 200 mg/kg (90.23 ±7.00) treated groups. Additionally, the SGOT in the diabetes control group increased Albizia lebback & comberetum indicum 200 mg/kg (72.00 ±6.00) treated group reduced greatly ($p< 0.001$), but the SGOT in the Albizia lebback & comberetum indicum 100 mg/kg (78.00± 600) treated group significantly fell ($p< 0.001$). According to Nagarajan et al. (2005), both the SGPT of the Albizia lebback & comberetum indicum 200 mg/kg treated group and the SGPT of the Albizia lebback & comberetum indicum 100 mg/kg treated group considerably decreased. Albizia lebback and Comberetum indicum significantly boosted the amounts of substances including glycosides, alkaloids, terpenoids, flavonoids, and others that are typically connected with antidiabetic properties when administered to diabetic rats. On their own, natural substances frequently have many pharmacological effects. These pharmacological multiactions are helpful criteria in the search for extremely potent drugs that can treat the complex symptoms of diabetes.

CONCLUSION:

Potential for efficient wound healing in Albizia lebback and Comberetum indicum Oral administration of Albizia lebback and Comberetum indicum at 100 and 200 mg/kg B.W shows good anti-diabetic and wound healing capabilities. However, the study need confirmation and more research. A metabolic condition known as diabetes mellitus (DM) is typified by poor glucose homeostasis brought on by abnormalities in insulin synthesis or activity. It is a widespread, possibly morbid disorder that has received a lot of medical attention. Understanding these types of illnesses requires the use of animal models. Diabetes arises in research animals either naturally or as a result of pharmacological, surgical, genetic, or other interventions. The illness displays a variety of clinical traits or phenotypes that are comparable to one another.

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