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ASSESSMENT OF THE WOUND HEALING EFFICACY OF PODOPHYLLOTOXIN IN STREPTOZOTOCIN – INDUCED DIABETIC RATS

Mr. Said Abdullahi Said, Ms. Arti Kumari, Ms Nidhi Sharma

Department of Pharmacy, School of Healthcare and Allied Sciences, G.D. Goenka. University, Sohna, Haryana, India.

Corresponding Author

Ms. Arti Kumari, Ms Nidhi Sharma

Assistant Professor

Department of Pharmacy, School of Healthcare and Allied Sciences, G.D. Goenka. University, Sohna, Haryana, India.

Abstract

The study and evaluated the wound healing activity of Podophyllotoxin in streptozotocin-induced diabetic in rats. Podophyllotoxin, a natural compound with known cytotoxic properties against wound healing enhanced, was examined for its potential to aid in wound healing, especially under diabetic conditions where wound healing is impaired. The research likely assessed parameters such as wound closure rate, histopathological biochemical changes, inflammatory response, and oxidative stress markers in diabetic animal models treated with Podophyllotoxin. The findings could provide insights into the therapeutic potential of Podophyllotoxin in managing diabetic wound healing complications.

Diabetes is a prevalent metabolic disorder that results in a delayed wound-healing process. Growing evidence proved that impaired glucose homeostasis is an independent risk factor for the occurrence of various types of diabetes including type 1 diabetes, type 2 diabetes, type 3 diabetes, and type 4 diabetes. Wound healing becomes the most severe complication of diabetic patients. Diabetes mellitus has a strong relationship with the healing process, thus there is a need to look for drugs that can be beneficial in maintaining glycemic levels as well as enhancing the process of healing. Therefore, more research is focusing on evaluating the role of antihyperglycemic agents in the wound-healing process. Metformin, sulfonylureas, and Insulin are the drugs that are used to enhance

the wound-healing process in diabetics. This Research gives a detailed overview of preparations of oinments, Thin Layer chromatography Analysis, phytochemical Screaning inducing of animals, weight of animals food consuming histopathology.

Introduction:

Wound healing refers to a living organism's replacement of destroyed or damaged tissue by newly produced tissue. In undamaged skin, the epidermis (surface, epithelial layer) and dermis (deeper, connective layer) form a protective barrier against the external environment. When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage. This process is divided into predictable phases: blood clotting (hemostasis), inflammation, tissue growth (cell proliferation), and tissue remodeling (maturation and cell differentiation). Blood clotting may be considered to be part of the inflammation stage instead of a separate stage (1).

Wound healing is a complex, dynamic process supported by a myriad of cellular events that must be tightly coordinated to efficiently repair damaged tissue. Derangement in wound-linked cellular behaviors, as occurs with diabetes and ageing, can lead to healing impairment and the formation of chronic, non-healing wounds(2).

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Skin wound healing starts immediately after injury and consists **of four phases:**

Phases of Healing:

Phases of Healing	**Days of post Injury**	**Cells involved in phase**
Hemostasis	Immediate	Platelets
Inflammation	Day 1 – 4	Neutrophils
Proliferation Granulation Contracture	Day 4- 21	Macrophages LymphocytesAngiocytesNeutrocytes Fibroblasts Keratinocytes
Remodeling	Day 21- 2 yrs	Fibrocytes

Haemostasis:

Immediately after injury, damaged blood vessels rapid contract and a blood clot forms preventing exsanguinations from vascular damage. Platelets, principle contributors to haemostasis and coagulation, are activated when they

encounter the vascular subendothelial matrix. Platelet receptors (e.g. glycoprotein VI) interact with extracellular matrix (ECM) proteins (e.g. fibronectin, collagen and von Willebrand factor), promoting adherence to the blood vessel wall. Thrombin subsequently triggers platelet activation, inducing a conformational change, and release of alpha and dense granules containing bioactive molecules which reinforce coagulation An insoluble clot (eschar) of fibrin, fibronectin, vitronectin and thrombospondin forms (3).

Inflammation

Innate inflammation evolved as the primary defence against pathogenic wound invasion. This immune response is initiated by injury-induced signals; damage-associated molecular patterns (DAMPs) released by necrotic cells and damaged tissue, and pathogen-associated molecular patterns (PAMPs) from bacterial components. These PAMPs and DAMPs activate resident immune cells, such as mast cells, Langerhans's cells, T cells and macrophages, by binding pattern recognition receptors to elicit downstream inflammatory pathways(4).

Proliferation

The proliferative phase of healing is characterized by extensive activation of keratinocytes, fibroblasts, macrophages and endothelial cells to orchestrate wound closure, matrix deposition and angiogenesis. As early as 12 h post-injury, keratinocytes are activated by changes in mechanical tension and electrical gradients, and exposure to hydrogen peroxide, pathogens, growth factors and cytokines .This activation causes keratinocytes at the wound edge to undergo partial epithelial–mesenchymal transition, where they develop a more invasive and migratory phenotype Front-to-rear polarity replaces top-to-bottom polarity, allowing the leading-edge keratinocytes to migrate laterally across the wound to reform the epidermal layer, a process termed re-epithelialization(5).

Matrix remodeling

Remodeling of the ECM spans the entire injury response, beginning with the initial deposition of a fibrin clot, and ending several years later with the formation of a mature, type I collagen-rich scar Fibroblasts are the major cell type responsible for wound ECM remodeling, replacing the initial fibrin clot with hyaluronic, fibronectin and proteoglycan, and forming mature collagen fibrils later in repair .Proteoglycans aid construction of mature, cross-linked collagen fibrils and act as a conduit for cell migration(6).

MATERIALS AND METHODS:

Plants of Podophyllotoxin were we purchacse the extracrion powder from the vital herbs campany in New Delhi. Autnification of Podophyllotoxin was done in New Delhi under the vitals herbs campany. Podophyllotoxin is a natural lignan-like compound with significant antitumor properties. The antidiabetic drugs etoposide and teniposide were synthesized with it as precursor compounds and have emerged as first-line chemotherapeutic agents for the treatment of many antidiabetic.

Thin layer chromatography (TLC) analysis

Thin layer chromatography of the samples of Podophyllotoxin methanol extract of P hexandrum was done using the solvent ethyl acetate and methanol (9:1) and the Rf value was calculated. RF value was 0.78.

RF = Distance travelled by the compound / Distance travelled by the solvent

RF = 2.2/2.8 = 0.78

Phytochemical screening

Phytochemical screening The Phytochemical screening of plant extract (Podophyllotoxin) samples was carried out by using the procedures of Kokate (1994) and Kokate et al (1995).

Detection of alkaloids

Mayer's test:

Mayer's reagent:

Wagner's test:

Wagner's reagent:

Detection of glycosides:

Detection of flavonoids:

Detection of volatiles

Detection of ash values: we take 4g of powder extract, put in to crucible and then weight, put in to muffle furnace at 450'0C, remove from muffle firnance after cooling the crucible then, weight of crucible.

Initial weight – final weight = Total ash value

26.6- 24.6= 2 ash value

Detection of swelling index: we take 50ml of measuring cylinder, then weight 1g powder extract put 25ml of water stir it 30-40 minutes.

Swelling index = volume of swollen material – original volume of liquid / weight of dry materials.

Swelling 12.5-25/30 = 0.41

Detection of foaming index: we take drugs 3mg and we add 100ml of water in cylinder we mix them. Height of the foam formed in the glass cylinder on tube using a rulers= 50.5.

Volume of the foam formed is 30.06.

Calculate by using formula.

Foaming index = Height or volume form / total height or volume x 100

50.05/ 30.06x100

= 16.8

Detection of Tannis: To 2 ml of extract 5 ml ethyl acetate and 2 ml conc H2 SO4 were added. Reddish brown coloration of interface indicated the presence of tannis and is 45%

Preparations of oinments:

In preparation of hydrophilic oinments of Podophyllotoxin: Stearyl alcohols and white petrolatum were melted together at about 75”c. The others agents including extracts in different concentration were dissolved in purified water are added with stirring until the mixture congeals. Sodium lauryl sulphates were act as emulsifying agents with Stearyl alcohols and white petrolatum comprising the oleaginous phase of emulsion and the other ingredients aqueous phase. At the last methyl and propyl paraben were added which is preservatives.

In preparation of hydrophobic oinments of Podophyllotoxin: The waxy bases were melted using water bath. Petroleum jelly which having high melting point were melted first. Then low melting substances cetostearyl alcohols, PEG 6000 and liquid paraffin were melted. After that different concentration of extracts were dissolved in small quantity of purified water and added it in large volume non- aqueous phase. At the last methyl paraben is added. Cooled under stirring and soft mass of oinments is obtained.

Procedure of ointment preparation:

Apparatus: Beaker, measuring cylinder, glass, stirring rod, thermometer, water bath, spatula, china dish.

Steps

Weight all the require ingredients of Podophyllotoxin cream properly and keep them seperatly

Take stearic acid and lanoline in a beaker and melt them at 60c.

Take another beaker and add glycerin, tnethonalamine, water and heat up to 60c. Then add the preparation of first beaker in a second beaker drop by drop with continuous stirring.

After cooling add methyl paraben, propyl paraben, roe oil, and mix them thoroughly to obtain uniform product.

Preparation of Podophylotoxin Ointment:

FORMULA	INGEDIENTS	AMOUNTS
5% W/W ointment preparation	Podophyllotoxin	1G
	Petroleum jelly	70g
	Cetostearyl alcohol	10g
	PEG Polyethylene glycol 6000	5g
	Liquid paraffin	10g
	Methyl paraben	5 drop
	Total = 100	

Animals:

Male Swiss albino rat weighing 180-250gm of either sex will be used in the study. Animals will be procured from Laboratory Animal House of GD. Goenka University. All animal experiments strictly complied with the approval of institutional animal ethical committee. The animals will be kept in polyacrylic cages and maintained under standard housing and feeding conditions. Approval for conducting the experiments in rats will be obtained from our Institional Animal Ethical Committee.

Induction of diabetic in rats:

The animals were injected by intraperitoneal route with a single dose of Streptozotocin (35) mg/kg, body weight) in normal saline. After 30 hours Fasting blood glucose level was measured by Accu check glucometer to confirm the diabetic status of the animals. The animals showing diabetes (Blood glucose level > 200 mg/dL) was selected for wound healing study using standard.

In vivo excision wound healing method in streptozotocin induced diabetics rats

Excision wound model:

Group	No. of Animals	Treatment
Group I (Normal)	5g	5 ml/kg/day distilled water, p.o

Group II (Standard)	5g	Streptozoocin (35 mg/kg, body weight) induced diabetes + Excision wound model (Diabetic wound standard Silverex Cream)
Group III (Control)	5g	Streptozotocin (35 mg/kg, body weight) induced diabetes + Excision wound model + 5% Silver Nitrate
Group IV (Treated group)	5g	Streptozotocin (35 mg/kg, body weight) induced diabetes + Excision wound model + 5 % Podophyllotoxin ointment

All surgical affairs were conducted under sterile condition. The rats were anaesthetized prior to development of the wounds and hairs from the dorsal thoracic central region of anaesthetized rats were removed followed by marking the area of the wound at the back of the animals. The wound was created along these markings by using toothed forceps, a surgical blade and pointed scissors. The thickness of the excision wound was 3 cm in width (circular area 4.0 cm2) and 0.2 cm depth which left open.

Phosphate buffer solution 0.4%

Prepare 500ml of Distilled water in a beaker. Add 20.2g of Sodium phosphate dibasic solution and add 10g of Sodium phosphate monobasic monohydrate to the solution and add 1ml of distilled water and stirred it. Then Take 4ml of formaldehyde in measuring cylinder and take solution of phosphate buffer 99% then mixed it 5-10 minutes and put it in Ph at 7.0

Tris HCL Solution of 99%

1. For a 10ML Solution, dissolve 12g of Tris HCL in 100ml of Distilled water in a beaker and mixed it.

2. Adjust the pH to 7.4 value by slowly adding approximately 6-7 mL concentrated Tris HCl. Adding concentrated HCl to the Tris buffer will increase the temperature of the solution, which affects the ph. Allow the solution to cool to room temperature before making final adjustments to the ph.

3. Adjust the volume of the solution to 100 mL with water.

4. Filter Tris-HCl with 0.22 µm sterile filter.

5. To obtain a 10 mM Tris-HCl pH 7.4 solution, dilute 1 M Tris-HCl pH 7.4 1:100 with nuclease-free water. For example, add 1 mL of 1 M Tris-HCl pH 7.4 to 99 mL of distilled water.

Histopathology:

Histological study of wound prior to animal sacrificed and wound tissue harvested, rats were euthanized by cervical dislocation under anesthesia using diethyl ether. Histological examinations were carried out to assess cellular responses and vascularization of wound tissue for further wound healing evaluation. Biopsies of wounded areas were performed using histological

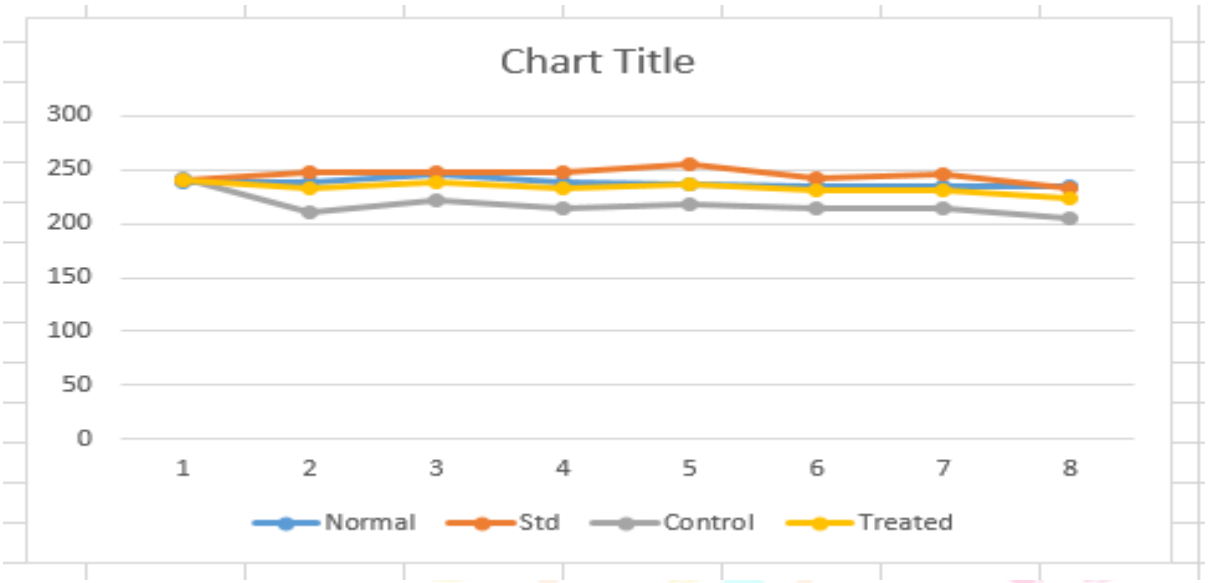
Evaluations 7 and 14 days post-wounding. The wound samples were fixed with a 10% formaldehyde buffer solution for 72 h. Samples then underwent a standard de-hydration process in a series of increasing ethanol concentrations for 24 h by a processing machine. Then, the tissue was degreased with xylene, embedded in paraffin, and section using a histological microtome. Sections of 5 thick tissue were mounted on a glass slide and stained using haematoxylin and eosin (H&E). This was followed the visualization of samples under a light microscope at100 x and 400 x magnification. A semi-quantitative method was used to examine the following histological structures and processes: epider-mal regeneration (100 x), granulation tissue thickness(100 x), fibroblast proliferation (400 x), angiogenesis(400 x) and the presence of inflammatory cells (400 x).Three stained sections from 10 random fields for each group were evaluated and scored using a scale of 1–3for epidermal regeneration and granulation tissue thickness; and a scale of 0–4 for fibroblast prolifera-tion, angiogenesis, and the presence of inflammatory cells as summarized in All stained images were taken by using microscope.

Result

Phytochemical Screaning

Extract constituents	**Present (+) or Absent (-)**
Alkaloid (mayers test, mayers reagent, wagners text, wagners reagent.	+ + +
Glycosides	+Blue, and green color appear
Flavonoids	+Green& Yellow color appear
Glycosides	+ Blue & Green color appear
Saponin	+ (Foams appears)
Steroids	- (no appearance observed)

Weight of Animal



Fiq.1

Weight of Animal- Normal and standard and treated its showing in the same level as increasing and the control it's showing the animal it's decreasing day by day. In this condition, the animal's weight remains relatively stable or shows a gradual increase over time. This suggests that under normal or standard conditions, the animal's weight either maintains a steady level or exhibits expected growth. This suggests that under certain treatments or experimental conditions, the animal's weight is decreasing. This could imply that the treatment is having a positive effect on the animal's growth or overall health.

Fig.2

Food consuming in this table normal and standard it is decreasing compare to control its showing a situation where food consumption in a table or dataset is decreasing in a normal or standard way compared to a control group or baseline. Here's how you might phrase it more clearly: The food consumption observed in this table shows a decreasing trend that is considered normal and standard when compared to **the control group.**

HISTOLOGY

Normal Group

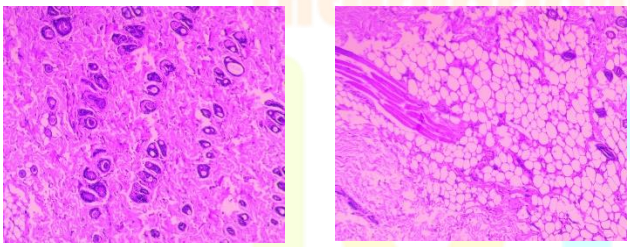


Fig:3 This show there is no injury occur or bound because its normal A normal histology result typically indicates that the tissue sample examined under a microscope appears healthy and shows no signs of disease or abnormalities. Histology involves the study of tissue structure and composition at a microscopic level, often used to diagnose or rule out diseases.

For example, if a biopsy of skin tissue shows normal histology, it means that the layers of the skin (epidermis, dermis, etc.) appear as expected with no signs of infection, inflammation, or abnormal cell growth (such as cancer).

Similarly, in the context of other organs or tissues, a normal histology result means that the cells and structures within the sample are within normal limits and do not suggest any pathology or disease process.

Standard Group

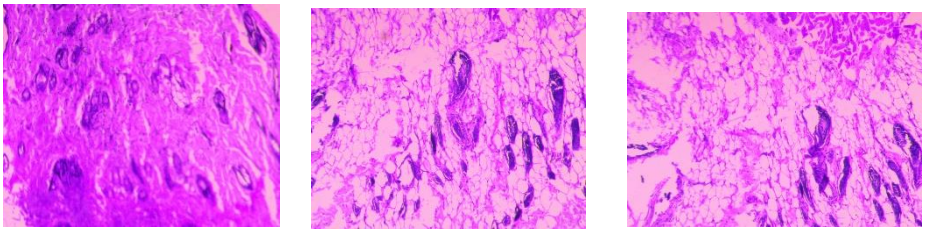


Fig: 4 Standard histology in microscopic images of tissue samples stained to highlight specific features. These images can show the structure of cells and tissues at various magnifications. May include annotations or labels to indicate different structures or cells within the tissue sample. These help readers understand what they are looking at and the significance of different parts of the image

Control Group

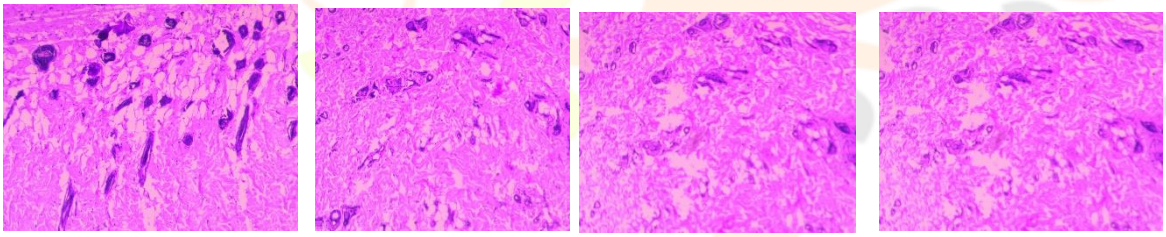


Fig:5 This fig show there is no any damage of the skin because we did not applied any creams and the control and the skin have heal properly than compare to standard.

Treated Group

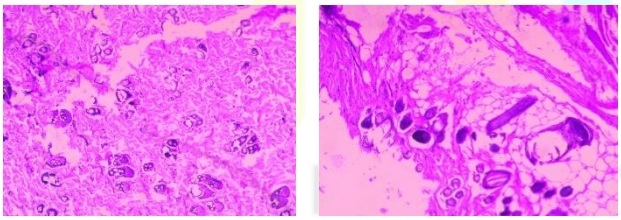


Fig. After treating the experimental group with a specific intervention, tissues (often biopsies or organ samples) are collected from these subjects. These tissues undergo histological processing, which includes fixation, embedding, sectioning, staining, and microscopic examination. The histological findings from the treated group are then compared with those from a control group. The control group typically consists of subjects who did not receive the treatment (or received a placebo) but were otherwise similar in other aspects.

Conclusion:

Diabetes is a prevalent metabolic disorder that results in a delayed wound-healing process. Growing evidence proved that impaired glucose homeostasis is an independent risk factor for the occurrence of various types of diabetes including type 1 diabetes, type 2 diabetes, type 3 diabetes, and type 4 diabetes. Wound healing becomes the most severe complication of diabetic patients. Diabetes mellitus has a strong relationship with the healing process, thus there is a need to look for drugs that can be beneficial in maintaining glycemic levels as well as enhancing the process of healing. Therefore, more research is focusing on evaluating the role of antihyperglycemic agents in the wound-healing process. Metformin, sulfonylureas, and Insulin are the drugs that are used to enhance the wound-healing process in diabetics. This Research gives a detailed overview of preparations of oinments, Thin Layer chromatography Analysis, phytochemical Screaning inducing of animals, weight of animals food consuming histopathology.

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