



Diabetic Wound Healing Potential of *Ocimum Sanctum* (Leaves) & *Santalum Album* (Bark) in Diabetic Rodent Model.

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ABSTRACT

Introduction: Impaired wound healing in diabetic patients without large vessel disease has been attributed to microvascular dysfunction, neuropathy, and abnormal cellular and inflammatory responses. The present study was aimed to develop a herbal formulation using herbs which has been proved to enhance the cell proliferation, cell migration and alleviate the inflammation and infection thus hasten the healing process. diabetic wound healing activity of herbal formulation containing two different extracts of *Ocimum sanctum* (leaves) & *Santalum album* (bark) powder by using aqueous extract cream. **Method:** Dried *Ocimum sanctum* (leaves) and *Santalum album* (bark) aqueous extracted by using distilled water. Albino rats were divided into four groups with five rats in each group. Alloxan monohydrate 2%, solution which was dissolved in 0.9% of sodium chloride (normal saline) as a diluent and given intraperitoneally to rats and blood glucose estimation made by using glucometer. Rats received alloxan at a dose of 150 mg/kg. Excision resutured wound models in albino rats was used to study complete epithelisation time, wound contraction of the wounds. **Results:** The time taken for 50% wound contraction and complete epithelisation by topical group 4 combination of *Santalum album* & *Ocimum Sanctum* extract with petroleum jelly as compared to Group 3 Combination of *Santalum album* extract & petroleum jelly. **Conclusion:** Topical combination of *Santalum album* & *Ocimum Sanctum* extract with petroleum jelly promoted better granulation tissue, early and complete epithelisation on rat compared to the control and standard drug group. The Phytochemical screening of the aqueous extract of *Ocimum sanctum* and *Santalum album* revealed the presence of flavanoid, tannins, saponins, steroids, phenols and alkaloids.

Keywords: - Anti-diabetic, *Ocimum sanctum*, *Santalum album*, alloxan, wound healing, Excision model.

INTRODUCTION

Diabetes is a major wellbeing problem universal as around 5% of the world's populace suffers from diabetes. Worldwide forecasts advise that more than 300 million persons will ensure diabetes by the year 2025 and the global cost of handling diabetes and its problem could reach US\$1 trillion annually. The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. Traditional plant drugs are used throughout the world for a range of diabetic performances. Therefore, study on such proxies from traditional healing plants has develop more vital. India has a rich history of spending various potent herbs and herbal gears for treating diabetes. Numerous Indian plants must be examined for their helpful use in various types of diabetes.

Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. For transferring of glucose, the hormone - insulin is needed which is mainly secreted by pancreatic beta cells. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major cause of morbidity and death.

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from deficiency in insulin secretion and its imbalance. The increased blood sugar level in diabetes mellitus is associated with long-term damage, dysfunction, and failure of various organs such the eyes, kidneys, nerves, heart, and blood vessels. As per 2016 data from the World Health Organization (WHO) shows the estimation of 422 million adults are living with diabetes mellitus world wide. The different types of diabetes are type 1 diabetes (diminished production of insulin) "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes", type 2 diabetes (impaired response to insulin and β -cell dysfunction) "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes" and the other is gestational diabetes which occurs during pregnancy. All type shows signs and symptoms like increase blood sugar level, excessive urine production, increased thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.

All though several drugs targeted for carbohydrate hydrolysing enzymes (pseudo saccharides), release of insulin from pancreatic β -cells (sulphonyl urea), glucose utilization (biguanides), insulin sensitizers are in clinical practise, the growing diabetes market observes a number of changes. Some of these

drugs are linked to liver toxicity (troglitazone), including number of deaths from hepatic failure and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone). The beneficial multiple activities like manipulating carbohydrate mechanism by various mechanism, preventing and restoring integrity and function of β -cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them in to novel therapeutics. The multifactorial pathogenicity of diabetes demands multimodal therapeutic approach. Thus, future therapeutic strategies require the combination of various types of multiple agents. Thus plant based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management.

In Indian medicinal system many plants are used for the treatment of diabetes. In Ayurveda, plant parts of different species have been used against diabetes. Medicinal plants provide a useful source of oral hypoglycaemic compounds for the development of new pharmaceutical drugs.

TYPES OF DIABETES MELLITUS

Diagnosis and classification of diabetes are performed according to certain criteria, and there are two main clinical entities. Diabetes mellitus is broadly classified, based on the clinical manifestations, into two main types such as type 1 diabetes (T1D) or insulin-dependent diabetes mellitus (IDDM) and type 2 diabetes (T2D) or non-2 insulin dependent diabetes mellitus (NIDDM). However, a number of authors have also recognized a third category called gestational diabetes or type 3 diabetes.

INSULIN RESISTANCE

Insulin resistance (IR) is a situation in which cells fail to react to the regular measures of the hormone insulin. The body produces insulin; however, the cells in the body develop into resistant to insulin and are incapable to use it as successfully, foremost to hyperglycaemia. Beta cells in the pancreas consequently increase their creation of insulin, additionally contributing to hyperinsulinemia. This repeatedly remains hidden and can donate to a diagnosis of type 2 diabetes.

A. Diabetic Nephropathy

Diabetic nephropathy is a general and severe complication everywhere kidneys are damaged and fails to function. The cause is due to unrelenting high blood sugar level in the blood. In the premature phase of nephropathy drugs along with diet can manage the condition. The situation while protein start leaking in urine is called as micro albuminuria. The general symptoms are kidney failure and weakness, decreased appetite, nausea and vomiting. Anaemia might also be identified in diabetic nephropathy.

B. Diabetic Neuropathy

Diabetic neuropathy is a complex disorder that impairs both peripheral and autonomic nervous system. Several possible pathogenic mechanisms have been proposed for diabetic neuropathy, but the pathogenesis still remains unclear suggesting that the overall mechanism could be complex and perhaps multifactorial. A number of vascular and metabolic factors have been implicated in the development of T2D-related neuropathy.

C. Diabetic Retinopathy

This is a microvascular complication that impairs normal function of the peripheral retina and/or the macula. It causes visual disability as well as partial or complete blindness in patients with T2D. The primary marker for this complication is the loss of pericytes which affect capillary constriction, new capillary generation and indeed other continuous exposure to noxious molecules.

CHRONIC DIABETES COMPLICATIONS

Diabetes is associated with certain long-term complications. These include microvascular complications typical for diabetes: retinopathy, nephropathy, and neuropathy. The macrovascular complications, e.g., stroke or heart disease due to enhanced atherosclerosis, however, are not specific to diabetes, but the risk of cardiovascular disease increases 3-8-fold in people with diabetes or impaired glucose tolerance. Furthermore, hypertension and dyslipidaemia are commonly coexisting with diabetes, and the patients are at risk of developing cardiovascular disease.

HYPERGLYCEMIA

Hyperglycaemia or **high blood sugar** is a state in which a too much quantity of glucose circulates in the blood plasma. This is normally a glucose level elevated than 11.1 mmol/l (200mg/dl).

- Polyuria - increased volume of urination
- Polyphagia- frequent hunger, especially pronounced hunger.
- Polydipsia - frequent thirst, especially excessive thirst
- Fatigue (sleepiness)

- blurred vision
- Dry mouth
- Weight loss

➤ **Drugs**

Some medications increase the risk of hyperglycaemia, including and Beta blockers, epinephrine Thiazide diuretics, niacin, pentamidine, protease inhibitors, Corticosteroids, octreotide Some Antipsychotic agents. The sensitive administrations of stimulants such as amphetamine typically produce hyperglycaemia. Various of the newer psychotropic medications, such while Olanzapine along with Duloxetine, can also cause considerable hyperglycaemia.

MEDICATIONS USED TO TREAT DIABETES

Historically, symptoms of diabetic diseases were recorded 3,500 years ago, in Egypt, Arabia, and Asia. Diabetes has been diagnosed and medication for managing diabetes mellitus was discovered in the 20th century. The discovery of diabetic remedies such as insulin in 1922, significantly reduced death in the diabetic population.³⁰ Since that time, the diabetic remedies and medications were greatly improved. Nowadays, the pharmacological controls of diabetes are more effective.

WHAT IS WOUND?

Wound healing is a process of restoring normal structure functions of damaged tissue. Healing is a natural phenomenon by which body itself overcome the damaged to the tissue, but the rate of healing is very slow and chance of microbial infection is high.[12] [17]

a. Acute wounds

Acute wound is a tissue injury that normally precedes through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time.

b. Chronic wounds

Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation chronic wounds either require a prolonged time to heal or recur frequently.

CLASSIFICATION OF WOUND

Wounds may be classified by several methods: their aetiology, location, type of injury or presenting symptoms, wound depth and tissue loss or clinical appearance of the wound. Wounds are classified as open and closed wound on the underlying cause of wound creation and acute and chronic wounds on the basis of physiology of wound healing.

WOUND HEALING ACTIVITY

This activity was performed as per the guidelines set by the Indian Science Academy, New Delhi, India. Twelve-week-old healthy wistar rats (150-200g) of either sex was used for the study. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" (IAEC) (Proposal. No. IAEC 20/2007) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules.

Wound healing is a dynamic, interactive process involving coagulation, inflammation, tissue formation, and tissue remodelling. Impaired skin wound healing is a common cause of morbidity and mortality among patients with diabetes.

WOUND HEALING IN DIABETES

The repair process is normally well-regulated with the timely and overlapping phases: inflammation, new tissue formation, and remodelling. Diabetes, however, delays this process. Treatments that enhance diabetic wound healing are often associated with reducing inflammatory cytokines in the diabetic wound environment.

Normal wound healing can be split into 4 Stages.

- Coagulation.
- Inflammation.

- Proliferation.
- Remodelling.

CAUSE OF DIABETIC WOUND

1. Increase Blood Sugar Level
2. Decrease or Poor Circulation
3. Peripheral Neuropathy /Nerve Damage
4. Immune System
5. Infection

TYPES OF DIABETIC WOUND

There are two most common diabetic wounds. They are external origin diabetic wounds and internal origin diabetic wounds. Due to peripheral neuropathy, wounds of external origin, such as skin cuts, burns, bumps and bruises, may often go unnoticed by the diabetic patient.

SIGNS & SYMPTOMS OF DIABETIC WOUND

Common signs and symptoms of diabetic wound are chronic pain or completely painless, signs of inflammation (swelling, redness, heat, pain and loss of function), signs of infection (pus drainage, discharge, bad odour and dead tissue), numbness and dullness (signs of nerve damage), fever and/or chills (signs of progressively worsening infection that can be limb threatening or even life-threatening).

PLANT DESCRIPTION

Ocimum sanctum :- *ocimum sanctum* L. commonly known as holy basil (Tulsi) is an herbaceous plant. It is considered as one of the most important sources of medicine and drugs with many secondary metabolites and essential oils recommended for treatment of malaria, diarrhoea, bronchial asthma, diseases etc. In addition, *Ocimum sanctum* also shows anticancerous, antifungal, antimicrobial, antifertility, hepatoprotective, antispasmodic, cardio protective, antiemetic, antidiabetic and analgesic properties.

Synonyms

English name - Holy basil/sacred basil

Hindi name - Tulsi

Sanskrit name - Tulsi

Gujarati name - Tulsi

Chemical Constituents

Medicinal plants are rich sources of different types of medicines and produce various bioactive molecules. Herbal plant extracts are very useful and are the major sources of medicine which play a vital role in controlling various types of pathogens and as growth promoters. The medicinal plants extract have now emerged as a good alternative as they are rich in a wide variety of secondary metabolites such as tannins, phenolics, alkaloids and flavonoids etc which enhance growth, innate immune response and disease resistance against pathogenic bacteria in human as well as in different organisms.

The medicinal plants are rich sources of secondary metabolites which are chemically and taxonomically extremely diverse compounds with obscure functions. Alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants and these antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: Sanctum

Santalum album

Santalum album is an evergreen tree that grows up to 20 m attaining girth of up to 2.4 m with slender drooping branch. Bark of the tree is tight, dark brown, reddish, dark grey or nearly black, smooth in young trees, rough with deep vertical cracks in older trees, red inside. fruit a globose, fleshy drupe; red, purple to black when ripe, about 1 cm in diameter, with hard ribbed endocarp and crowned with a scar, almost stalkless, smooth, single seeded.

Powdered heartwood is used to make incense sticks, burnt as perfumes in houses and temples, or is ground into a paste and used as a cosmetic. The bark contains about 12-14% tannin and has good potential in the tanning industry.

Classification

Kingdom : plantae

Clade : Tracheophytes

Clade : Angiosperms

Clade : Eudicots

Order : Santalales

Family : Santalaceae

Genus: Santalum

Species: S.album

PHARMACOLOGICAL ACTIVITIES

Pharmacological studies have established that sandalwood and its root bark possessed abortifacient, hepato protective, urinary antiseptic, stomachic, anti-viral and anti-herpetic activities. The hydrolyzed exhausted sandalwood powder (HESP) possesses anti remorogenic, anti-inflammatory, anti-mitotic, antiviral, anti-cancerous, anti-hypertensive, anti-pyretic, sedative, ganglionic blocking and insecticidal properties.

MATERIAL AND METHOD

Plant material :- Plant *Ocimum sanctum* (leaves) & *Santalum album* (bark) collected from jainnursery Indore. leaves & bark of the plant washed with running water, dried in shade at room temperature, ground to powder and stored in air tight bag in dry at room temperature.

Preparations of Extracts

***Ocimum Sanctum* :-**

Preparation of aqueous extract of *Ocimum sanctum* (leaves) - The extract of leaves was obtained in sufficient quantity by using distilled water. In this process firstly 200 g powdered leaves of *Ocimum sanctum* were placed in 1000 ml of beaker and 800 ml of distilled water was poured into beaker after addition of water kept for overnight at the room temperature approximately 22 hrs for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent then, extract was filtered by using muslin cloth followed by Whatman no 1 filter paper then the green colour filtrate was obtained. Then the extract is dried in a digital water bath till a dark green residue were obtained.

***Santalum album* :-** The wood of *Santalum album* L. is ground to thick paste using little quantity of water. 100 gm of paste are taken in 250ml of water. It was kept overnight, and filter next day using muslin cloth. Then the extract is dried in a digital water bath. Phytochemical screening of the aqueous extract is carried out for the presence of various phytoconstituents qualitatively.

Phytochemical testing

1. Test for alkaloids

- **Mayer's test-** 5 mg extract of *Ocimum Sanctum* (Tulsi) was transferred in the test tube and then added 1% hydrochloric acid HCl, the obtained solution was gently heated. Red colour indicate the presence of alkaloids because Potassium mercuric iodine are present in Mayer's reagent.
- **Wagner's test-** In this test 5 mg extract of *ocimum sanctum* was taken in a test tube than 0.5 of Wagner reagent was added in a solution Shaked well. Appearance of reddishbrown colour showing the alkaloids are present. Reddish brown colour because of iodine forms a complex is insoluble and has the colour brown reddish.

2. Test for flavonoids

- **Shinoda test:** Firstly, 5mg extract was added in the test tube then small amount of magnesium was mixed in this solution, also added the few drops of concentrated Hydrochloric acid. It should be indicate the pink colour with the flavonoids. Colours varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavanones. Catechins when treated with vanillin solution in hydrochloric acid give red, pink colour.

3. Test for glycoside

- **Liebermann's test-** Liebermann' test for the analysis of glycoside are present or not in aqueous extract of ocimum sanctum in this test 5 mg extract of ocimum sanctum was mixed properly with 2ml of chloroform and then 2ml of acetic acid were mixed in the. Solution than it was cooled in ice. After cooling 1 ml of concentrated Sulfuric acid was added. The colour will be change from violet to green with the presence of alkaloids in the extract.
- **Salkowski's test-** for the analysis of glycoside 2ml of chloroform were with 1ml of extract. Then 2ml of concentrated Sulfuric acid were added and shaken gently.

4. Test for tannins

- **Ferric chloride test-** 5 mg aqueous extract of *Ocimum sanctum* was mixed with 0.5 ml of ferric chloride solution. Formation of blackish precipitate in the presence of tannin.
- **Test for saponins-** Foam test was performed for identify cation of saponin in the aqueous extract in which 1ml extract was dissolved into the 5ml of distilled water. After addition of distilled water, it was shaken for proper mixing till foam was observed. Few foams was added with 2 drops of olive oil and it was shaken vigorously. It should be produced emulsion with the saponins.

5. Test for oil

- **Stain test-** few quantity of aqueous extract was spread onto the filter paper formation of oil on the filter paper will indicate the presence of oil in aqueous.

6. Test for carbohydrates

- **Benedict's test-** Benedict's reagent was taken for the analysis of carbohydrate. the 5 mg extract was mixed with few drops of benedict's reagent, then allowed to boiled, the reddishbrown precipitate are found with the presence of the carbohydrates (absent).

7. Test for steroids- 5 mg extract of ocimum sanctum was mixed with 1 ml of chloroform then few drops of concentrated Sulfuric acid and acetic acid were added into it. The greenish colour was indicate the presence of steroids.

- **Salkowski's test-** 3 drops of concentrated sulphuric acid was added into the 5 mg extract. The formation of red colour indicates the presence of steroids.

8. Test for proteins

- **Biuret's test-** 5 mg extract was added with the few drops of biuret's reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins.

Alloxan induced diabetic rats-Animals were divided into four groups with five rats in each group. Total of 20 healthy male albino rats weighing 150-200 g were included in the study. The methods employed in this study to induce diabetes was chemical method using alloxan monohydrate 2%, solution which was dissolved in 0.9% of sodium chloride (normal saline) as a diluent given intraperitoneally at dose of 150mg/kg BW to rats and blood glucose estimation made by using glucometer. Fasting blood glucose levels was measured after alloxan administration at 24, 48 and 72 hours by using glucometer. The rats were given 5% (w/v) glucose solution in feeding bottles for next 24 h in their cages to prevent hypoglycaemia after alloxan injection. After 72 h rats with Blood glucose level (BGL) greater than 200 mg/dl and less than 400 mg/dl were selected and observed for hyperglycaemia (fasting blood glucose level –FBG) greater than 200 mg/dl and lesser then 400 mg/dl) up to 7 days.

Animal grouping

animals were divided into four groups as follows: each group contain 5 animals.

Group 1 Normal control (Vehicle petroleum jelly)

Group 2 Standard (T bact ointment)

Group 3 Combination of Santalum album extract & petroleum jelly

Group 4 combination of Santalum album & ocimum Sanctum extract with petroleum jelly.

Wound Models Under anaesthesia surgical applications of skin tissue wound models were performed. Dorsal aspect of each anaesthetic animal was shaved then wound area was cleaned by using ethanol (70 % (v/v)).

Excision wound model

For monitoring wound contraction and wound closure time, the open excision-type wound was created by biopsy punch on the dorsal interscapular region of the each rat with 1.5 cm diameter in a circular manner with the removal of skin. The excisional wounds were left open after surgical applications. After stitching wound was left undressed then standard ointment (T bact) and combination of santalum album (extract) & petroleum jelly ointment 3% , combination of santalum album & ocimum sanctum extract with petroleum jelly 3% ointment were applied daily up to 10days, when wounds were cured thoroughly the sutures were removed on the 10 day and wound contraction was measured using scale and visual appearance.

RESULT & DISCUSSION

Table 1. Phytochemical of Ocimum test sanctum(leaves) &Santalum album(bark)

Test Name	Ocimum sanctum	Santalum album
Alkaloid Wagner's Test	+ve	+ve
Carbohydrates Benedict's Test	-ve	+ve
Glycoside Liebermann's Test	-ve	-ve
Flavonoids Shinoda Test	+ve	+ve
Tannins Ferric chloride Test	+ve	+ve
Saponine Foam Test	+ve	-ve
Oil Test Stain Test	-ve	-ve
Proteins Biuret Test	-ve	-ve
Steroids Salkowski's Test	-ve	-ve

Preparation of ointment

ointment was prepared by mixing accurately weighed Ocimum sanctum (leaves)powder and Santalum album (bark) powder extract to the ointment base by aqueous extract method and add petroleum jelly for thickness and blend with the help of motor pistol to prepare a smooth paste.gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.

Wound healing activity on diabetic rats

Table 2 Effect of extract ointment Santalum album &Ocimum sanctum on excision wound model

S.No.	Treatment	Period of epithelization	% wound contraction on 2 nd day	% wound contraction on 4 th day	% wound contraction on 6 th day	% wound contraction on 8 th day	% wound contraction on 10 th day
1.	Control	15 days	13.4±1.5%	26.4±1.4%	31.2±0.7%	39.2±1.1%	45±1.8%
2.	Standard	8 days	16.5±1.4%	28±2.0%	38.7±1.8%	47±1.5%	58.2±1.3%
3.	SA with petroleum jelly (Test I)	13days	11.4±1.7%	16.5±1.2%	22.9±1.8%	28±1.5%	37.5±1.5%
4.	SA+OS+ Petroleum jelly (Test II)	9 days	12.7±1.3%	19.4±0.3%	31.9±1.3%	39.9±1.5%	50.1±1.7%**

results are expressed as mean \pm sem.* $p > 0.05$, ** $p < 0.01$ as compared to control

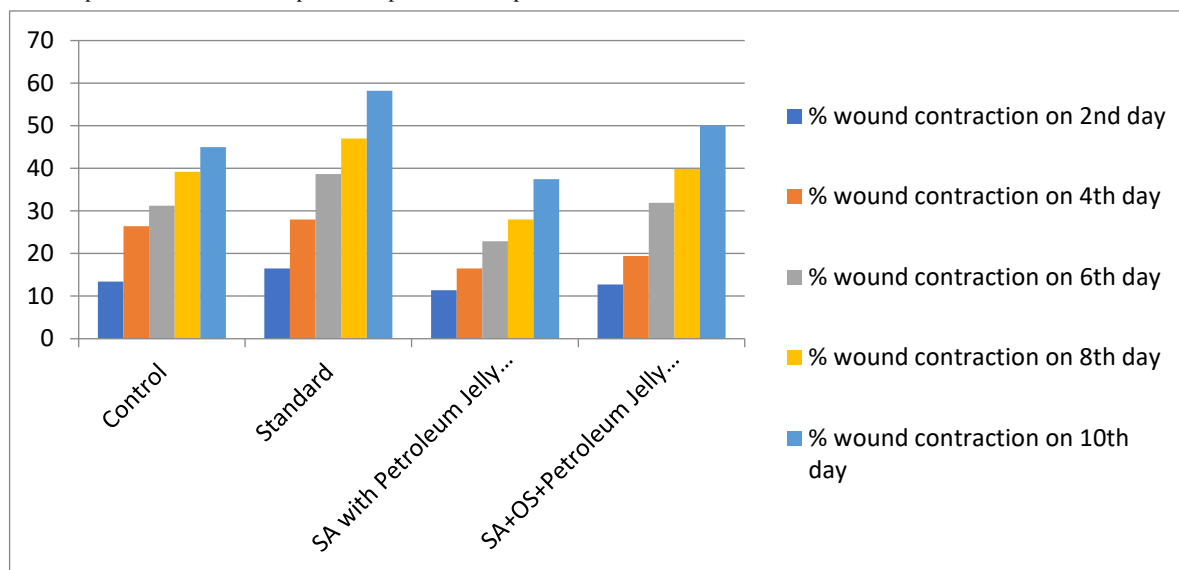


Figure 1 Wound diameter in different groups of diabetic and control rat after application of *santalum album* & *ocimum sanctum* ointment.

Conclusion

It is concluded from this study that the extract of plant *Ocimum sanctum* & *Santalum album* possess significant diabetic wound healing potential, which substantiates the traditional use the whole plant of *Ocimum sanctum* & *Santalum album* in the treatment of different diabetic as well as non-diabetic wound.

The result of the study show significant wound healing in diabetic rodent model % wound contraction found to be (by *Santalum album* and petroleum jelly extract ointment is - 37.5%.

Combination of (*Santalum album* + *Ocimum sanctum* + petroleum jelly) extract ointment of show better result as compared to the *Santalum album* and *Ocimum Sanctum* extract ointment % wound contraction found to be 50.1 %. On 10th day.

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