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Evaluation of Preliminary Phytochemical Analysis of Operculina Turpethum Roots Extract for Diabetic Wound Healing Activity in Rat

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Abstract

Operculina turpethum linn has been traditionally claimed that the roots of Operculina turpethum linn are useful in the management of wound healing. The roots of Operculina turpethum linn are reported to have anti-inflammatory, antioxidant, and antimicrobial properties. In the present study, wound healing activity of ethanolic extracts of Operculina turpethum linn was evaluated in rats using excision wound model along with estimation of phytochemical parameters. In the excision wound model, the period of epithelisation, scar area, percent wound contraction or closure was evaluated respectively. The treatment of diabetic wound with oral containing ethanolic extracts of Operculina turpethum linn exhibited significant wound healing. The results were comparable to standard drug povidone iodine ointment, in terms of percentage of wound contraction, parameters. A significant antioxidant activity was observed as evidenced from decreased level of MDA and increased the level of GSH, catalase in extract treated animals as compared to control animals.

Keywords: Wound healing, *Operculina turpethum*, Excision Diabetic wound, Phytochemical Screening.

Introduction

Long-term consequences of diabetes include the occurrence of foot ulcers and decreased wound healing(1). Changes in the inflammation related growth factors and course of inflammation in the wound have been linked to these problems, according to studies(2-4). Inflammation and wound healing are facilitated by cells from the monocyte-macrophage system. The function of cells are altered by Diabetes (5,6). Furthermore, chronic hyperglycemia has been linked to a reduced rate of foot ulcer healing(7). The aim of this

study was to see how a macrophage stimulant affected inflammation and wound healing.

Inflammation, new tissue creation, and remodeling are all aspects of the repair process that occur in a timely and overlapping manner (8). However, diabetes slows down this process. Inflammatory cells (macrophages, B-cells, and plasma cells) were found in biopsies taken from chronic diabetic ulcers, a decrease in CD4 (*Clusters of differentiation 4*)$^{+}$ T cells, there is no evidence of migration or epidermal growth across the surface of wound, and blood vessels constriction within the lesion's edge (2,4). Diabetic mice and rats, on the other hand, have much fewer inflammatory cells in their wounds than non-diabetic animals(3,4,9). Extrinsic and intrinsic variables cause healing impairment in diabetics (neuropathy, vascular issues, and other diabetes-related systemic complications) (wound infection, excessive pressure and callus formation, to the site) (10).

Operculina turpethum linn., synonym Ipomoea turpethum, has been used as ayurvedic medicine for the treatment of a variety of diseases since ancient times, including fevers, ascites, edoema, , constipation, anorexia, haemorrhoids, hepatosplenomegaly, abdominal tumours, intoxication, fistula, anaemia, obesity. *Operculina turpethum* linn. is a threatened species that needs to be protected right now. (11-12).

The root bark contains 10 % glycosidium resin. It also contains turoethin, a glycoside that gives it purgative properties. It also contains two glycosides, volatile oils, and a yellowish material.

Material And Methods

Collection of plant:

In the month of July 2021, roots were procured from sellers of herbal plants in Bhopal, M.P., and brought to the local market of Bhopal. The root portion was removed and thoroughly cleaned.

Ethical Committee Clearance

Ethical clearance was taken from the animal ethics committee of the Institute (726/02/B/CPCSEA/21).

Extraction of Operculina turpethum linn:-

Preparation of extract:

Extraction with petroleum ether (40-60°) was performed to remove lipids, followed by extraction with ethanol in a soxhlet extractor. The roots extract was concentrated under a vacuum to obtain the residue. Vacuum desiccators were used to dry the residue. Ethanol's extractive yield was discovered to be higher, hence it was chosen for wound healing activities. The vehicle (200 mg w/v acacia mucilage) was used to make all of the test suspensions (200 mg/ml), which were then administered orally at a dose of 200 mg/kg. Each time, the petroleum ether-treated medication was air-dried before being extracted with alcohol. Fresh, untreated medication was used to make the aqueous extract (11).

After a successful roots extraction, the solvent was concentrated at room temperature and under reduced pressure in a rotary evaporator, and the water content was removed in the water bath. The consistency and color of the extract were noticed.

Drying and size reduction of plant material:

The roots of *Operculina turpethum* linn. was washed and cut into manageable pieces

and allowed to air dry. Then it had a physical assessment with a range of characteristics. As evaluation criteria, size, shape, width, and length from nature were all used.

Finally, the root was size reduced to obtain a coarse powder, which was then sieved no. 40 to obtain a homogenous powder. The homogenous powder was then exposed to different parameters of standardisation.

Preliminary phytochemical analysis of crude extract: - (12)

- To detect phytoconstituents, various qualitative tests were performed on the extracts obtained by solvent extraction.
- **Tests for alkaloids:-**
- **Dragendorff's test:** 1 ml of extract treated with ragendorff's reagent (potassium bismuth iodide solution). Alkaloids are present when an orange-red precipitate forms.
- **Mayer's test:** Mayer's reagent was used to treat 1 ml of extract (Potassium mercuric iodide solution). Alkaloids are present when a whitish-yellow or cream-colored precipitate forms.
- **Hagers's test:** Some drops of hager's reagent treated with 2-3 ml of filtrates yield yellow-colored ppt.
- **Wagner's test:** 2-3 mL of filtrate treated with wagner's reagent yields a reddish-brown ppt.
- **Tests for glycosides: -**

Determined the extract's free sugar content and hydrolyzed it with mineral acid (di. HCL/dil. H2SO4). Use the hydrolyzed extract test to ascertain the amount of sugar overall. The presence of glycosides is indicated by an increase in sugar concentration in the extract.

HOH
Glycoside ⟶ Aglycon (genin) + Glycon (suger)

- **Test for cardiac glycoside:-**
- **Baljet's test**: 1ml of the extract coloured yellow to orange when combined with 1ml of sodium picrate solution, showing the presence of glycosides..
- **Legal's test:** To make the extract alkaline, dissolve it in pyridine and treat it with sodium nitroprusside solution. The presence of glycosides is indicated by the formation of pink to red colour.
- **Test for Deoxy sugars (Keller-Killiani test):** 2 mL of extract treated with glacial acetic acid, 1 drop of 200 mg Fecl2 and concentrated sulfuric acid was mixed. Where the two liquid layers meet, a reddish-brown tint is visible.
- **Tests for Carbohydrates: -**
- **Molisch's test (General test):** Separately, a small amount of the extracts were taken and dissolved in ethanol and a few drops of 20% w/v solution of α-naphthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates.
- **Test for Reducing sugar:**
- **Benedict's test:** 1ml extract solution mixed with 5ml Benedict's reagent, boil for 2 minutes, then cool. The presence of sugars is indicated by the formation of a red precipitate.
- **Test for Monosaccharides:**
- **Barfoed's test:** The extract and Barfoed's reagent were mixed in equal parts. Red ppt is seen after being heated for two minutes in a boiling water bath and then cooling.
- **Tests for Steroids:**

- **Salkowski's test:** An equivalent volume of conc. H2SO4 was added to the extract after it was dissolved in chloroform. The steroidal components in the examined extract are represented by the chloroform layer producing a bluish-red to cherry color and the acid layer producing green fluorescence.
- **Liebermann-Burchard test:** Small amount of each extracts treated with 1 ml of acetic anhydride into the test tube, after that sulfuric acid was applied by the side to each test tube. The presence of sterols was indicated by the appearance of blue color after the contents had cooled.

➢ **Test for Proteins and Amino acids:**

- **Biuret test (General test):** 1ml of the extract treated with 2 drops of 1 percent CuSO4 solution and 1ml of a 40 percent sodium hydroxide solution until a blue color appears. appear pinkish or purple; the presence of proteins is indicated by the color violet..
- **Millions test:** 5 ml of millions reagent mixed with the extract. Appeared white precipitate in solution, warm the solution precipitate turns brick red in color.
- **Ninhydrin test: (General test):** 3 mL of test solution heated + 3 drops 200mg ninhydrin solution The colour purple or bluish appears..

➢ **Tests for Saponins:**

- **Foam test:** 1 cc of distilled water and a tiny portion of the extracts were heated and stirred. Saponins were present, as evidenced by the development of foam.
- **Hemolytic test:** On a glass slide, a drug extract or dry powder is treated with a single drop of blood. There is a hemolytic zone.

➢ **Tests for Tannins:**

- **Ferric chloride test:** Separately, a small fraction of each extract was dissolved in 2 ml of distilled water and filtered. The filtrate has been examined with a solution of ferric chloride. The presence of tannins was indicated by the appearance of a blue to bluish-green or bluish-black color
- **Gelatin test:** a small amount of extractis added to gelatin. The appearance of white ppt. represents the presence of tannins.

➢ **Tests for Flavonoids:**

- **Shinoda test:** After being extract treated with a few drops of magnesium and strong hydrochloric acid, a pink scarlet, crimson red, or occasionally a green to blue tint appeared.
- **Alkaline reagent test:** Test solution turns a vivid yellow color when some drops of sodium hydroxide solution was mixed and turns colorless when some drops of diluted acid was mixed. (13)

➢ **Test for fat and oil**

(a) A thick section of the extract was placed on a glass slide, treated with a drop of Sudan III reagent. Observed under a microscope. An oil globule appears.

(b) To thin sections treated with 1% osmic acid after 1min., observed under a microscope. Oil globule appears.

Preliminary pharmacological screening of potent roots extract for diabetic wound healing potential in rats followed by bioactivity guided fractionation *(13,14,15,16)*

➢ **Procurement of experimental animals:** BRNCP Mandsaur animal house provided Wistar rats weighing between 100 and 150 g of either sex. The mice were stabilized for one week in twelve hours light-dark cycle and kept in a typical environment of room temperature, 60-200mg relative humidity, and a. They were fed with a regular pellet meal and had access to unlimited water throughout experiment. The animals

were handled gently The animals were handled gently to avoid stressing them out, that could lead to an increase in adrenal output.

Diabetic wound healing activity evaluated in rats inducing by the followings methods:

- Streptozotocin (90 mg/kg body weight)
- Alloxan (120 mg/kg, i.p.)

➢ **Excision wound model (300 mm ^{2}and 2 mm depth) Developing condition:** The rate of wound contraction and epithelization were studied using excision wounds. All of the lesions were full-thickness, going all the way to the adipose tissue. Diethyl ether vapour inhalation was used to sedate the rats, and each rat's right side was shaved**.** Excision Cutting away a portion of skin from the shaved area resulted in wounds measuring 300 mm 2 and 2 mm in depth. The wound was completely left open. The rats were constantly monitored for signs of infection if any one shown infection sign were separated and replaced from the trial. In all of the cases, the therapy was applied topically. For 16 days, the extract was administered. A transparency sheet and a permanent marker was used to measure the wound of all group on days 1, 4, 8, and 16. On graph paper, the wound regions were measured. The day on which the scar fell after wounding but before there was any remaining raw wound was designated the epithelization day.

Diabetic wound healing evaluation parameters

Wound contraction and time: To assess the wound healing, wound contraction was measured. In Group IV, significant wound contraction began on day 12 ($p < 0.05$). From day 20, wound contraction was highly significant in Groups IV and V ($p < 0.01$), as well as Group III.

As shown, the epithelialization time was dramatically shortened in Groups III, IV, and -V ($p < 0.05$). Treatments with latex contained in hydrogels, as well as traditional burn-treatment cream, outperformed the untreated and gel-treated groups.

Result & Discussion

Extraction: Ethanol was used as a solvent to extract the plant's dry powder. To produce the extract, the solvents were removed via low-pressure distillation, and a rotary flash evaporator was used to vacuum dry the resulting semisolid mass. Table:1 shows the % yields of various extracts, while Table:2 shows the findings of a preliminary phytochemical investigation.

Table 1: *Pharmacognostical Extractive value of extract*

Sr. No.	Extract	Nature of extract	Colour of extract	% Yield (w/w)
I	Alcohol	Semisolid viscous	Brown	14.641
II	Aqueous	Semisolid viscous	Brown	12.542

Table 2:- *Preliminary phytochemical analysis results*

Sr. No.	Name of the Test	Observation	
		Alcoholic Extract	Aqueous Extract
I.	**Tests for Sterols**		
	• Salkowski's Test	+	-
	• LibermannBurchard's Test	+	-
II.	**Test for Glycosides**		
	• Baljet's Test	+	+
	• Keller–Killiani Test	+	+
	• Legal's Test	+	+
III.	**Tests for Saponins**		
	• Foam Test	+	+
	• Haemolysis Test	+	+
IV.	**Test for Carbohydrates**		
	• Molish's Test	+	+
	• Barfoed's Test	+	+
	• Benedict's Test	+	+
V.	**Tests for Alkaloids**		
	• Mayer's Test.	-	-
	• Wagner's Test.	-	-
	• Dragendorff's Test	-	-
	• Hager's Test	-	-
VI.	**Tests for Flavonoids**		
	• Ferric chloride Test.	+	+
	• Shinoda Test.	+	+
	• Alkaline Reagent Test.	+	+
	• Lead Acetate Test.	+	+
VII.	**Tests for Tannins**		
	• Ferric chloride Test.	-	-
	• Gelatin Test	-	-
VIII.	**Test for Amino acid and Protein**		
	• Million's Test	-	-
	• Ninhydrin's Test	-	-
IX.	**Test for fixed oils and fat**	-	-

(+) indicate positive result (-) indicate negative result.

Study design of Operculina turpethum linn in wound healing

The rats were divided into four groups, each group having six rat.
Group I - Treated with normal saline
Group II- Assigned as Diabetic Control
Group III- Assigned as Povidine Iodine
Group IV- Assigned as root Aquous Extracts 1ml/kg (200mg/kg)
Group V - Assigned as root Ethanolic Extract 1ml/kg (200mg/kg)

Table 3. *Effect of Operculina turpethum linn seed extract on percent wound contraction*

Drugs	Percentage of closure of excision wound after 15 days						Incision wound
Group	**0 Day**	**3rd Day**	**6th Day**	**9th Day**	**12th Day**	**15th Day**	**18th Day**
I NC	385.2	323.3	318.1	280.4	223.3	110.5	80.5
II DC	388.4	354.2	340.3	288.5	260.3	205.7	110.5
III PI	385.6	343.6	304.3	208.3	86.6	32.3	00
IVOTAEO (200mg)	382.3	341.0	291.7	186.6	186	20.3	10.3
V OTEEO (200mg)	382.6	341.8	287.6	147.3	61.6	15.3	5.1

't' test p<0.0200mg at various day variance in all groups

Statistical Analysis

A one-way ANOVA analysis of variance and Dunnette's multiple comparison test was used to determine the statistical significance. Mean, SEM, and P0.05 are used to express the values. Each value is expressed as follows: mean SD, n=3, NC=normal control, DC=Diabetic control, PI=Povidone Iodine, OTAEO=*Operculina terpenthum* linn Aqueous Extract Oral, and OTEEO=*Operculina Terpenthum* linn Ethanolic Extract Oral.

Like the excision wound model, the application of alcoholic extract *Operculina turpethum* linn (200mg), Both oral and topically applied povidone Iodine 5% shortened the epithelization period significantly (P <0.001) and also produced a significant reduction (P <0.001) in wound contraction-50% (days) as compared to control. When compared to low doses of *Operculina turpethum* linn and standard, the *Operculina turpethum* linn at high dose to be more effective. The extract and suspension of *Operculina turpethum* linn were found to have significant (P<0.05) wound healing in all excision models.

Summary & Conclusion

The topical administration of *Operculina turpethum* linn extract has a good effect on skin wound healing, according to our findings. In diabetic rats, the therapy induced a faster and more efficient cutaneous healing when used in preparations containing 200mg of *Operculina turpethum* linn extract. The topical treatment's ability to stimulate cellularity, TGF levels, collagen, and elastic fibre deposition, as well as attenuate oxidative damage in scar tissue, accelerated wound closure, was linked to the healing benefits.

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