Role of Medical Plant in Wound Healing Activity: An Ethnopharmacology Analysis

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Abstract

The study of ethnopharmacology and traditional medicine has made drug development feasible in the worldwide pharmacopoeia in the present environment. The current study provides a scientific rationale for the traditional use of Catharanthus roseus L. leaves in the treatment of diabetes and delayed wound healing in diabetic mice, demonstrating that they effectively stimulate insulin secretion and promote wound contraction in diabetic mice when compared to control and other drug-treated groups. This study shows that the plant's extract may be evaluated for anti-diabetes and wound-healing capabilities in humans, and that it might be utilised commercially to treat diabetic wounds on a big scale.
KEYWORD: Diabetes; Wound Healing; Plant; Medicine

1. INTRODUCTION

Ethnobotany

Ethnobotany is a crucial area of study and acknowledgeable all over the world. This science shows healthy relationship between humans and nature and provides possibility of finding new uses of medicinal plants and can be utilized to discover new drugs derived from plants (Heinrich M et al., 2004). This interdisciplinary science, of late, has incorporated into it the varied faculties of anthropology, sociology, archaeology, forestry, medicine folklore etc., thus has gained further importance. Conventional medical methods such as Chinese, Ayurvedic, Unani, and Biomedicine are quite efficient in treating many disorders, especially those living in rural.

In the study of plant-human relations, it is obvious that inter- and transdisciplinary methods can lead to more successful, comprehensive, and systematic approximations. Ethnobotany is an intriguing and promising field of research, despite or maybe because of its numerous hurdles (Alexiades NM., 1996). Ethnobotany may be grouped into three major categories:

- Quantitative ethnobotany
- Applied ethnobotany
- Ethnoecology

Concept Of Diabetes & Wound Healing

Diabetes is a set of metabolic illnesses marked by hyperglycemia caused by insulin secretion abnormalities. Diabetes' persistent hyperglycemia is linked to long-term damage, malfunction, and failure of several organs, including the eyes, kidneys, nerves, heart, and blood vessels. Diabetes is caused by a number of different pathogenic mechanisms. These can range from autoimmune death of the pancreas' P-cells, resulting in insulin shortage, to anomalies that lead to insulin resistance. Diabetes causes anomalies in glucose, lipid, and protein metabolism due to insulin's ineffective action on target tissues. The world health organization recognizes three main types of diabetes mellitus:

- Type - 1 Diabetes (IDDM, Insulin dependent diabetes mellitus)
- Type - 2 Diabetes (NIDDM, Non-Insulin dependent diabetes mellitus)
- Gestational diabetes (Occuring during Pregnancy)

Mechanism Of Wound Healing

Wound healing is a complicated process that occurs after an injury to the skin or affected organs (Nayak BS et al., 2007). The epidermis, the outermost layer of the skin, and the dermis, the inner or deeper layer, are in steady-state equilibrium in normal conditions and constitute a protective barrier against the external environment. If this protective barrier is breached as a result of an injury, the regular physiological wound healing process begins right away. When the skin is injured, a series of complicated biochemical reactions occur in a well-ordered sequence to repair the damage. Platelets consolidate at the injury site within minutes of the damage, forming a fibrin clot. The purpose of this clot is to stop aggressive bleeding and establish hemostasis. The full wound healing process, which begins at the time of damage, might take months or even years to complete. There are three main phases of wound healing represented in Table.l. These phases are as following:

- Inflammatory phase
- Proliferative phase
- Remodeling phase

The research conducted so far for combating diabetic ulcers have mainly focused on control of infections by the use of antibiotics and dressings. These are just able to control the inflammation superficially without combating the actual cause.

Objectives of The Study:

The main objectives of the research is the role of Medical plant in wound healing activity: An Ethnopharmacology analysis.

2. MATERIALS AND METHODS

Due to its geographical and climatic conditions, the forest region of Maharashtra in India is one of the biggest source of medicinal plant variety. For its ethnobotanical merits and therapeutic relevance, the Gondia district in Maharashtra, India, is one of the least explored places.

Interviews with 54 people aged 45 to 70 who possessed traditional plant knowledge were conducted to gather information. Cotton defined semi-structured interviews as the strategy for collecting ethnobotanical data (1996).

Ethnobotanical data was analyzed and summarized using Microsoft Excel and statistics to determine frequency of citations in order to identify the most common ailments in the study area, commonly used medicinal plant species, and multipurpose plant species, and to determine proportions of various variables such as plant families, growth forms, source of collection, degree of scarcity, plant part used, methods of preparation, and threatening factors.

3. EXPERIMENTAL PLANTS

A number of medicinal plants have been reported for their ethnomedicinal importance in the treatment of different kinds of diseases by tribal peoples from gondia, district. In which Catharanthus roseus L. was selected for their antidiabetic and wound healing activity.

Catharanthus roseus L. (Sadahahar)

Catharanthus roseus L leaves were taken then dried the material, powdered it, and stored it in airtight containers. Fresh plant material was gathered in bulk after verification, rinsed under running tap water to remove clinging material, dried in the shade, and ground in a mechanical grinder. The coarse powder was sieved no. 40 and taken for further investigation.

Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) using SNK test (Students-Newmann-Keuls) with sigma state 3.5. The p-value less than 0.05 were considered to be significant (Level of significance P<0.05=*, P<0.01=**, P0.001=***). Data were represented as mean ± standard deviation. All the studies were performed in quadruplet.

5. DATA ANALYSIS AND RESULTS

The data presented are the overall findings of an ethnobotanical study done in the forest region of gondia district, Maharashtra, India. The indigenous inhabitants of this area used 40 different plant species from 27 different families. There were 11 herbs, 15 shrubs, 8 trees, and 6 climbers among them. Liliaceae (5), Fabaceae (4), and Solanaceae (4) were the most regularly represented families (3). The findings of a growth form analysis of medicinal plants revealed that shrubs had the largest proportion (15 species, 37.50%), followed by herbs (11 species, 27.50%), trees (8 species, 20.00%), and climbers (8 species, 20.00%). (6 species, 15.00 percent). This observation is in contrast to the typical trend found in most medicinal inventories (Giday et al., 2003; Giday et al., 2007), which shows that herbaceous medicinal plants are the most common.

Of the 40 medicinal plants, 33 species (82.48%) were collected from the wild while 4 species (10.00%) were found in cultivation and 3 species (7.50%) were obtained both from cultivation and the wild. This shows that practitioners receive medicinal plants from wild sources or the natural environment rather than from home gardens, and medicinal plant cultivation is quite limited in the research region. It also suggests that traditional healers are overusing the gondia district's natural forest for its therapeutic plant composition.

Roots, stems, leaves, and other plant components are commonly utilized to treat human and cattle health issues. Roots (33.91 percent) and leaves (33.91 percent) were the most often employed plant components for herbal remedies in the area (25.65 percent). Such widespread root harvesting, which is necessary for plant life, has a detrimental impact on the survival and continuity of beneficial medicinal plants, and hence on their long-term usage. Addis et al. (2001) identified a large percentage of herbal prescriptions from root sources in their ethnobotanical research. The ethnomedicinal importance of some of the individual medicinal plants found in the Gondia district utilized by indigenous peoples and practitioners is studied here.

4.PHARMACOLOGICAL ACTIVITY OF THE LEAVES OF CATHARANTHUS ROSEUS

4.1. Qualitative And Quantitative Analysis Of The Phytochemicals In The Leaves Of Catharanthus Roseus

Qualitative and quantitative analysis of the leaves of Catharanthus roseus revealed the presence of secondary metabolites flavonoids, phenols, saponins, alkaloids, tannins and primary metabolites carbohydrates, proteins, lipids. Quantitative estimations of bioactive constituents are summarized in Table. 4.1 .Catharanthus roseus has been tremendously used in the treatment of several diseases.

Table. 4.1: Qualitative and Quantitative analysis of the leaves of Catharanthus roseus

Bioactive constituents	Presence	Quantity in gram % (w/w)
Carbohydrate	+	0.31±0.04
Alkaloids	+	2.11 ±0.51
Steroids	_	_

Saponins	+	3.11±1.01
Tannins	+	0.67±0.75
Flavonoids	+	1.71±0.91
Phenols	+	1.59±0.43
Lipids	+	2.27±0.59
Proteins	+	0.81±0.81

*Results are mean ± SD of quadruplet determination on the basis of dry weight.

4.2. ANTIDIABETIC ACTIVITY' OF THE LEAVES OF CATHARANTHUS ROSEUS

Administration of Catharanthus roseus leaves as drugs resulted in a significant decrease (P<0.001) in serum glucose level on 3rd, 7lh and 13th day in case of diabetic mice as compared to the diabetic controls. A less significant decrease (P<0.05) was observed in case of mice treated with metformin on day 7 but on day 13 this decrease was highly significant (PO.OOl) mice [Table. 4.2].

Table. 4.2: Effect of Catharanthus roseus leaves on the level of serum glucose (mg/dl) in various diabetic and nondiabetic groups of mice.

Groups	**Level of serum glucose (mg/dl)**			
	0th Day	**3rd Day**	**7th Day**	**13th Day**
NDC	103±2.23	101±2.16	98±2.86	96±2.65
NDM	108±2.11	105±2.41	102±3.18	93±2.18
NDM+E1	107±2.42	104±2.72	102±2.60	95±2.89
ND+E1	111±1.92	107±2.19	99±2.19	91±2.73
ND+E2	106±2.87	102±2.32	94±2.43	88±2.73
DC	222±3.12	228±2.56	236±2.37	264±1.70
DM	207±2.76	181±2.28	155±1.93	142±2.98
DM+E1	211±2.66	176±2.13	142±2.71	118±2.62
D+E1	219±2.50	171±2.57	138±2.53	112±2.29
D+E2	216±1.99	167±2.08	132±2.31	108±2.31

4.3 WOUND HEALING ACTIVITY OF THE LEAVES OF CATHARANTHUS ROSEUS

The measurements of the wound diameter were taken on the day 3rd, 7th and 13th using transparency paper and a permanent marker. The results are summarized in Table. 4.3.

Table. 4.3: Wound diameter (mm2) in different groups of diabetic and non-diabetic mice after the administration of Catharanthus roseus leaves as antidiabetic drugs.

Groups	Wound contraction (mm2)		
Groups	3rd Day	7th Day	13th Day
NDC	69.12±0.92	38.96±1.01	3.28±1.33
NDM	61.73±0.72	32.17±0.99	0.37±1.25
NDM+E1	56.39±1.12	39.75±1.15	2.11±1.49
ND+E1	77.91±0.98	48.21±1.35	0.76±1.58
ND+E2	70.11±1.23	51.45±1.52	0.49±1.31
DC	86.23±0.93	81.32±1.32	52.34±1.28
DM	91.35±1.18	61.26±1.76	2272±1.19
DM+E1	89.41±0.88	83.61±1.22	24.32±0.92

D+E1	82.67±1.31	66.45±1.34	15.23±1.36
D+E2	79.32±0.94	62.87±1.19	9.12±1.54

On graph paper, the wound regions were documented and measured. In diabetic mice, the leaves of Catharanthus roseus accelerated wound healing. In the D+E_2 diabetic group, wound closure was excellent (Figure.4.6).

4.4 BIOCHEMICAL ESTIMATION OF SOD, LPO & NO IN GRANULOMA TISSUE AFTER ADMINISTRATION OF THE LEAVES EXTRACT OF CATHARANTHUS ROSEUS

Levels Of SOD In Wound Tissue After Drugs Administration

The wound tissue from diabetic mice showed decreased extra cellular SOD activity as compared to non-diabetic mice as shown in [Table. 4.4].

Table. 4.4: Level of SOD (% inhibition of SBT reduction ml of wound tissue supernatant) in various diabetic and non-diabetic groups of mice after administration of Catharanthus roseus

Groups	**Level of SOD**		
Groups	**3rd Day**	**7th Day**	**13th Day**
NDC	2.03±0.09	2.10±0.07	2.23±0.11
NDM	1.96±0.02	1.99±0.18	2.07±0.11
NDM+E1	1.81±0.12	1.89±0.1	1.93±0.11
ND+E1	1.77±0.13	1.95±0.14	2.08±0.15
ND+E2	1.73±0.19	1.98±0.16	2.10±0.17
DC	1.02±0.08	1.17±0.11	1.56±0.12
DM	1.73±0.11	1.76±0.04	1.97±0.14
DM+E1	1.81±0.13	1.93±0.08	2.03±0.11
D+E1	1.98±0.14	2.13±0.16	2.45±0.18
D+E2	2.12±0.16	2.28±0.2	2.75±0.12

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After administration of Catharanthus roseus leaves as drugs, the SOD activity was found significantly increased on both 7lh and I3lh day in all diabetic groups injected with plant extract ($p<0.05$). Ilowever no significant changes observed in non-diabetic groups.

Levels of LPO in woundtissue after drugs administration:

The estimated amounts of lipid oxidation in wound tissue supernatant from diabetic and non-diabetic plant drug treated groups of mice were as shown in [Table. I6| and [Table. 4.5] (Figure. 4.8). When comparing diabetes wounds to non-diabetic wounds, the level of LPO in terms of MDA (nmol/ml) was higher. Catharanthus roseus leaves were shown to be beneficial in avoiding lipid peroxidation, as evidenced by a reduction in malondialdehyde levels in diabetic groups. In non-diabetics, the drop in LPO was not substantial.

Table.4.5: Level of lipid peroxidation in terms of malondialdehyde (nmol ini of wound tissue supernatant) in various diabetic and non-diabetic groups of mice after administration of Catharanthus roseus

Groups	***Level of LPO***		
Groups	***3rd Day***	***7th Day***	***13th Day***
NDC	69.45±2.92	66.23±3.08	62.1±3.07
NDM	76.67±3.11	72.45±3.67	68.03±3.54

NDM+E1	63.12±3.49	59.32±2.86	51.89±3.54
ND+E1	57.61±3.22	56.5±2.65	44.76±2.92
ND+E2	60.09±2.99	52.17±2.87	41.2±2.54
DC	107.02±4.67	92.41±3.92	78.35±2.91
DM	85.06±2.75	72.7±3.24	53.2±2.34
DM+E1	79.53±3.12	68.3±3.13	50.21±3.24
D+E1	74.18±2.88	58.7±1.99	41.04±2.54
D+E2	66.65±2.24	48.12±2.93	32.43±3.11

Levels of NO in wound tissue after drug administration

The wound tissue from diabetic mice showed decreased nitric oxide level as compared to non- diabetic mice [Table. 4.6]. After administration of Catharanthus roseus leaves as drugs, the NO was found increased on both 7,h and 13th day in all diabetic groups beings significant ($p<0.05$).

Table. 4.6: Level of total nitric oxide content (pM) in wound tissue supernatant in various diabetic and non-diabetic groups of mice after administration of Catharanthus roseus

Groups	Level of NOX(µM)		
Groups	3rd Day	7th Day	13th Day
NDC	35.04±1.98	38.62±2.18	42.09±1.96
NDM	31.87±2.13	34.76±2.22	44.11±1.99
NDM+E1	36.52±1.87	41.17±1.65	47.32±2.31
ND+E1	38.81±1.76	43.32±2.98	49.83±1.96
ND+E2	33.28±2.32	47.01±1.89	51.13±2.35
DC	40.57±2.29	51.19±2.63	58.72±2.11
DM	39.04±2.43	56.92±2.11	64.87±2.39
DM+E1	43.07±2.56	59.01±1.74	68.33±2.09
D+E1	47.51±1.92	61.22±1.88	71.19±2.92
D+E2	49.78±2.09	65.56±2.23	77.09±2.19

Therefore, it is clear that the plants Catharanthus roseus showing the similar activity in both diabetic and non-diabetic mice. It also support the evidence of other researchers on the same and others plants. Wound healing activity of the leaves of Catharanthus roseus Intraperitoneal injection of Catharanthus roseus extract resulted in enhances wound healing in diabetic mice. In term of reduction of wound area in thirteen days as compare to control the wound closure was found to be maximum in the mice treated with 400 mg/kg close to Catharanthus roseus extract. The wound healing potential of this herbal extract could be attributed to the high content of alkaloids, flavonoids, and tannins which impart astringent and antiseptic properties responsible for increased rate of cpithelialization which ultimately result in wound contraction.

The wound healing also correlated with the efficient management of oxidative stress by increased SOD and NO level due to Catharanthus roseus extract. As a phytoconstituent (phenolic and tannins) to possess strong LPO inhibition and free radical scavenging abilities they increase the level of SOD and NO and help in prompt wound repair by enhancing the transport of oxygen towards the wound and by promoting various other cellular repair mechanisms. This might be the reason that the extract has been used since long time as a potent wound healing agent by the traditional and tribal communities.

5. DISCUSSION

Qualitative and quantitative analysis of Catharanthus roseus

The presence of tannins, triterpenoids, and alkaloids was discovered during a preliminary phytochemical examination of the leaves extract. Any of the phytochemical elements found in Catharanthus roseus might be to blame for the wound healing ability. Tannins aid wound healing through a variety of biological mechanisms. Chelating free radicals and reactive oxygen species, enhancing wound contraction and increasing capillary channel and fibroblast development Recent research has revealed that phytochemical elements such as flavanoids and triterpenoids improve wound healing, owing to their astringent and antibacterial qualities, which appear to be responsible for wound contraction and higher epithelialization rates. Catharanthus roseus' wound-healing ability might be linked to the phytoconstituents found in the plant, and the faster wound healing process could be due to the individual or cumulative actions of the phytoconstituents. The tannin phytoconstituent of Catharanthus roseus, which has an astringent effect, may have contributed to the early tissue approximation and higher tensile strength of the excision wound seen in our study (Chaudhari M et al., 2006). Earlier studies showed the presence of triterpenoids which are responsible for the effective wound healing activity of Cecropiapeltata (Nayak BS., 2006) and Pentas lanceolata (Nayak BS et al., 2006).

Antidiabetic activity of Catharanthus roseus

Administration of Catharanthus roseus leaf extract resulted in highly reduced glucose level on the seventh and thirteenth day of treatment. The effect of extract was more obvious and visible in diabetic mice as compared to the metformin treated diabetic mice. Besides, the addition of the Catharanthus roseus leaf extract of metformin as a therapeutic enhances the potential of the synthetic drug as shown in the results. Moreover, there is not much differences in the hypoglycemic effect when the dose is increased from 200 mg/kg to 400mg/kg, suggesting that the 200mg/kg may be given as the optimum dose. The hypoglycemic effect on this plant could be explained in term of its richness in secondary metabolites such as alkaloids, saponins, flavonoids, phenolic and tannins. Which have been proved to enhance insulin secretion and regeneration of P- cell of islets of langerhans in case of diabetic patients.

Wound healing activity of the leaves of Catharanthus roseus

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6. CONCLUSION

The ethnobotanical findings described in this study give actual proof of medicinal plant use among the gondia district residents of Maharashtra, India. Furthermore, the data demonstrated that the area's medicinal plants represent a key supply of herbal medications for rural people' primary health care. The current study provides a scientific rationale for the traditional use of Catharanthus roseus L. leaves in the treatment of diabetes and delayed wound healing in diabetic mice, demonstrating that they effectively stimulate insulin secretion and promote wound contraction in diabetic mice when compared to control and other drug-treated groups. This study shows that the plant's extract may be evaluated for anti-diabetes and wound-healing capabilities in humans, and that it might be utilised commercially to treat diabetic wounds on a big scale.

Catharanthus roseus extracts exhibited strong hypoglycemic effect as their administration into the diabetic mice resulted in drastic decrease in blood glucose levels. The extract was found to be even more effective than the standard drug metformin. These plants contain high alkaloids, flavonoids, tannins and phenolics content which are proved to increase the insulin resistance and regenerate the pancreatic P-cell. It can thus be predicted that these secondary metabolites could be responsible for the hypoglycemic activities of these plants. Besides, both the plant showed fast wound healing potential in terms of reduction of wound area. This could be correlated with the increase in increase in SOD and NO levels and inhibition of LPO caused by the herbal extract which in turn minimizes the oxidative stress in the wounded mice and thus helps tissue regeneration faster.

The results clearly indicate the high ethnopharmacological significance of Catharanthus roseus and that can be used as effective and safe alternative medicines either singly or in combination with the synthetic drugs for the management of diabetes and associated complications like delayed wound healing.

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