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Original Research Article

Biochemical evaluation of depigmentation in C57/BL6 mice and its treatment by Psoralea corylifolia. Linn (Bakuchi) seed oil and seed extracts

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ABSTRACT

Skin is the largest organ of the body it protects us from several kinds of environmental hazards and also works as a connective unit between environment and individual. Skin can react in several ways against emotional factors. Alopecia areata and vitiligo are such diseases which effect individuals and their social environments. Vitiligo is a depigmented disorder where complete loss of melanocytes takes place. There are six major factors which define aetiology of vitiligo. Present study is an endeavour to establish a treatment which can change the painful allopathic treatment process of UV exposure, which results in post treatment hazards such as psores, inflammation, and pain. In the present study C57/BL6 mice were selected. The groups are divided in protocol 1; control, toxicant a and toxicant b, protocol 2; 100 mg/kg b.w, 200 mg/kg b.w and 300 mg/kg b.w of ethanolic extract of Psoraleya corylifolia. Linn seeds, and 100 mg/kg b.w, 200 mg/kg b.w and 300 mg/kg b.w of petrolium ether extract of Psoraleya corylifolia. Linn seeds. Topically applied groups of Psoraleya corylifolia Linn. seed oil for 3 weeks, 5 weeks and 7 weeks. The last group was petrolium ether 100 mg/kg b.w+ seed oil topically applied for 5 weeks. Further SOD (superoxide dis mutase), GSH (reduced glutathione), GPx (glutathione per oxidase), MDA (Malone di aldehyde), vitamin E were estimated on the depigmented skin samples. The study concluded that petrolium ether 100 mg/kg b.w showed better results for SOD and MDA, while for GSH and vitamin E ethanolic extract 300 mg/kg b.w showed better results in comparisons to the earlier one. The study successfully justified the hypothesis and the animals were relaxed during the study.

1. Introduction

Vitiligo is an acquired, idiopathic, hypomelanotic disease characterized by circumscribed depigmented macules (Ontonne J P and Bose S K., 1993)[1] vitiligo is a disorder of pigmentation where the loss of functional melanocytes takes place. Vitiligo affects 1% of the population. According to recently updated literature prevalence of vitiligo persists from 0.5 -2% of the total world population (Bergqvist C and Ezzedine K., 2020).[2] This problem is best emphasized by the terminology used in southern India as ven kushtham, meaning white leprosy (Gupta et al., 2012).[3]

Clinical presentation includes vitiligo, characterized by a lesion that occurs in a) dermatomal, asymmetric distribution of limited clinical significance b) focal vitiligo, characterized by a limited number of small lesions c) generalized vitiligo, the most common type of vitiligo. Where lesions occur in bilateral symmetrical distribution and d) universal vitiligo, complete or almost complete depigmentation (Gupta et al., 2012; Boisseau- Garsuad et al., 2002).[4]

16-35% of patients with vitiligo experience psychiatric morbidity. Depression 10%, dysthymia (17-19%), sleep disturbance (20%), suicidal thoughts (10%), suicidal

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attempts (3.3%), and anxiety (3.3%) have been found in those affected with vitiligo. It can also lead to difficulties in forming a relationship, avoidance of certain social situations, and difficulties in sexual relationships (Porter J et al.,1990; Ongenae K et al., 2003).[4] Vitiligo affects the patients psychologically which is well recognized (Elbuluk and Ezzedeni., 2017).[5] Because appearance is quite important in the present day to day life, and easily visible skin disorder affects the mental status and daily life of the patient pushing the patient towards the daily stigma which results in mental disorder (Wu and Cohen., 2019).[6] There are three main hypotheses for the pathogenesis of vitiligo: self-destruction, neural and autoimmune.

1.1. Biochemical basis of vitiligo

Melanocytes are melanin-producing cells found in the skin. Melanocytes are highly dendritic and these dendrites project into the Malpighian layer of the epidermis where they transfer the melanosomes to approximately 36 keratinocytes in the neighborhood and this entire unit is called the epidermal melanin unit (Shahjil et al.,2006). Apart from skin, melanocytes are present in retinal pigment epithelium, uveal tract, inner ear, and leptomeninges. In they reside in the matrix of the hair follicle of the basal layer of the epidermis. Tyrosinase is a key enzyme required for melanin synthesis. Tyrosinase catalyzes the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA), which is the rate-limiting step of melanin synthesis (Hearing VJ.,1999).[7] DOPA undergoes oxidation of dopaquinone, which is immediately converted to DOPAchrome and then to 5,6 dihydroxy indole (DHI). TRP2 (tyrosine-related protein 2) converts dopachrome to dihydroxyindole carboxylic acid (DHICA). DHI and DHICA further polymerize to form eumelanin. Cystine/ glutathione reacts with dopaquinone to produce cysteinyldopas that undergo further cyclization to benzothiazines and higher condensates give rise to confer photoprotection to the skin from ionization radiations (Hearing V.J., 1999).[7] Keratinocytes are the cell where the storage of melanin takes place. Human keratinocytes are the cells that make up the majority of the epidermis and express only beta 2 adrenergic receptors. The beta-adrenergic receptor is a seven-pass trans-membrane G protein-linked coupled receptor. A beta 2 AR expression is more highly expressed at the basal layer of the epidermis and decreases in expression toward the stratum corneum. Both keratinocytes and melanocytes express beta 2 AR, and keratinocytes have been demonstrated to generate norepinephrine. Stimulation of the beta 2 AR on melanocytes increases the intracellular level of cAMP and subsequently increases melanogenesis. Normal epidermal keratinocytes can endogenously generate epinephrine, they may be provided local stimulation of this beta 2 AR-mediated pathway for melanogenesis in the normal melanocytes.

Psoralen the active component of Psoralea corylifolia Linn. is recommended by clinicians, but the mode of action inside the body is exactly not known. It's not a permanent treatment. During the therapy, the patient is exposed to UVA and UVB light for at least half an hour. This causes heavy inflammation, pain, and sores on the exposed skin. UVA and UVB exposure can also cause melanoma. It is not justified ethically, to cure a disease-causing another disease. This treatment is not a reliable treatment and time taking. And the patient has to suffer more and more stress and social trauma, because of disturbed family and social life. The seeds are more effective than the psoralen (active component). Other chemical components of Psoralea corylifolia Linn. having potent antioxidant potential, will also be studied to get better results. The objectives of the present study were comparative evaluation of different doses of different extracts of Psoralea corylifolia seed and seed oil in the model system Rattus rattus species C57/BL6 through the biochemical study of different pigment makers. To study the rate of regeneration of the apoptotic melanocytes in the skin by increasing the antioxidant potential and avoiding the inhibition of tyrosinase the rate-limiting enzyme of melanogenesis. To establish the drug and its doses for the treatment of vitiligo. To study the changes occurring in depigmented skin by histopathology and recovery via Psoralea corylifolia seed and seed oil administration.

1.2. Hypothesis

1. Psoralen in the active component of Psoraliya corylifolia Linn. seeds, it is applied topically on the affected area, under the UV exposure for minimum 15 minutes, which results in trauma, pain, inflammation and psores in the patients. The treatment also revert after therapy completion. The present study is designed by providing the seed extract of Psoralia corylifolia Linn. with seed powder orally and seed oil topically without any UV exposure only in the presence of sunlight. No psores, inflammation or uneasiness was observed in the study samples, due to increased anti-oxidant potential.
2. Increased anti- oxidant potential played a major role in regeneration of melanocytes.

2. Materials and Methods

The study was carried out in the Department of Biochemistry in collaboration with the Department of Pharmacology, G. R. Medical College, Gwalior (M.P). The approval of the ethical committee has been taken for this research work. The herbal sample (seeds) of Psolarea corylifolia. Linn has been collected from Lalitpur district. U.P and the samples were authenticated by Ayurvedic Research Centre, Gwalior (M.P).

C57/BL6 strain of mice was selected for the present study. The mice were purchased from National Centre for Laboratory Animal and Science (NCLAS), Hyderabad. Ten mice were purchased and further breeding was carried out under lab conditions. 12/12 hrs day-night cycle, with water from Ad Labitum and protein-rich food pallets, were provided. Complete care of echogenicity was taken. SOD, MDA, GSH, GPx, vitamin E, and uric acid were estimated in skin punch lysate. In the acute study control group and two toxicant groups were taken. The control group has non-vitiligo mice, while toxicants a and b have 5%gm w/v TBC and 10%gm w/v TBC topically. In subchronic study 100 mg/kg b.w, 200 mg/kg b.w and 300 mg/kg b.w ethanolic extract and petroleum ether extracts of Psoralea corylifolia seeds given orally.

3. Results

A total of 78 mice were taken for the study. In the control group, healthy or non-vitiligo mice were studied. Animals were divided into 13 groups of 6 mice each. The toxicant groups were studied to gain the best toxicant. 5% gm w/v TBC in acetone has further proceeded for the study. Significant change of study parameters in drug-treated groups (group 4to group 9), by 6 different doses of ethanolic and petroleum ether extract 100 mg/kg b.w, 200 mg/kg b.w, and 300 mg/kg b.w. 100 mg/kg b.w of petroleum ether extract showed a significant increment in SOD (P<0.01**) and a less significant increment in vitamin E (P<0.05*). But no significant changes were found in the level of MDA, GSH, GPx, and uric acid (Table 2).

Significant changes were seen in the study parameters in drug-treated groups topically by seed oil for 3 weeks, 5 weeks, and 7 weeks. Group treated topically by seed oil of P. corylifolia for 5 weeks, showed significant lowering (P<0.01**) in SOD level in comparison to toxicant a group and less significant increment.

(P<0.05*) in vitamin E level. Highly significant (P<0.01**) increment was seen in GPx level in 5 weeks seed oil treatment group. No significant change in the level of MDA, GSH, and uric acid (Table 3)

The Table 1 shows levels of SOD, MDA, GSH, GPx, Vitamin E and Uric acid in group 1 to group 3, each having 6 animals.

Table 2 showing levels of SOD, MDA, GSH, GPx, Vitamin E and Uric acid in group 7 to group 12, each having 6 animals.

The Table 3 shows levels of SOD, MDA, GSH, GPx, Vitamin E and Uric acid in group 10 to group 12, each having 6 animals.

The Table 4 shows levels of SOD, MDA, GSH, GPx, Vitamin E and Uric acid in group 1, group 2, group 7 and group 13, each having 6 animals.

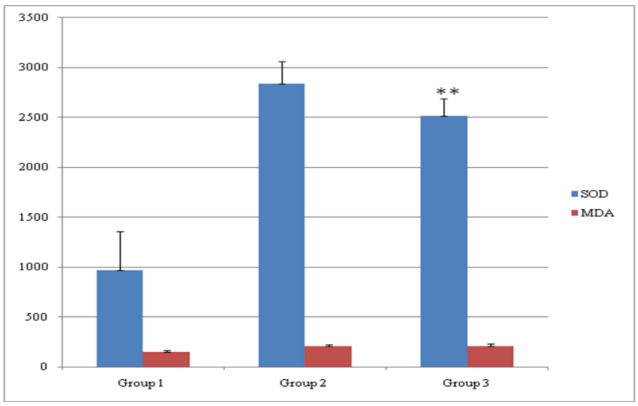

Fig. 1: Showing graph of SOD and MDA in groups 1 to 3

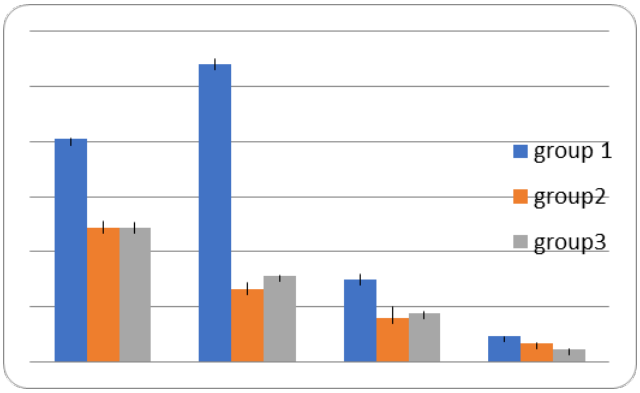

Fig. 2: Showing graph of GSH, GPx, Vitamin E and uric acid in groups 1 to 3

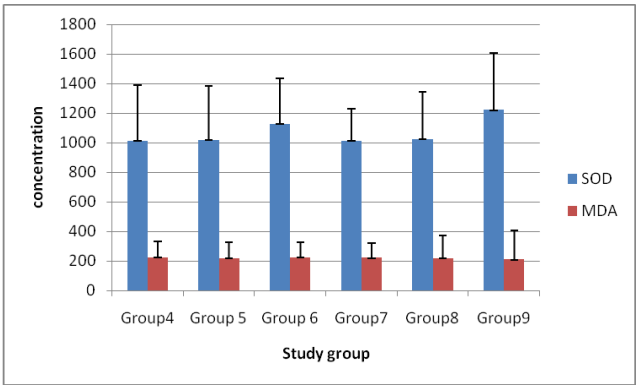

Fig. 3: Showing graphs of SOD and MDA in groups 4 to 9

Table 1: Study parameters of SOD, MDA, GSH, GPx, vitamin E and uric acid

S. No	Group 1 (n= 6)	Group 2 (n= 6)	Group 3 (n= 6)
SOD (U/ ml)	969.15±388.56	2840±201.60	2515±156.27**
MDA (mole/gm/min)	151±13.01	210±13.26	205±29.27
GSH (mg/dl)	40.41±0.12	24.25±0.96	24.25±1.09
GPx (μmole GSH utilized)	54.00±1.20	13.23±1.23	15.52±1.17
Vitamin E (IU/ mole plasma)	15.00± 1.04	8.00±1.40*	8.82±1.90
Uric acid (mg/dl)	4.56± 1.07	3.34±1.80	2.26±1.09

Table 2: Showing study parameters in group 4 to 9

S. No	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
SOD(U/ml)	1015.09±376.09	1019.12±375.10	1126.12± 380.12	1012.12±275.08	1026.14±371.12	1221.12±360.12**
MDA (mole/gm)	225.01±21.10	220±22.07	225.12±21.08	221.02±21.10	220±20.12	210±20.14
GSH (mg/dl)	42.26±1.14	39.24±1.804	40.25±1.04	46.02±1.14	46.02±1.18	46.01±1.14
GPx (μ mole/GSH utilized)	41.02±1.39	40.09±1.17	45.10±1.18	45.03±1.16	48.04±0.98	44.0±1.18
Vit E (IU/ mole hom)	11.03±1.50	12.06±1.68	14.07±1.66	12.05±1.68	12.07±1.61	11.08±1.60
Uric acid (mg/dl)	4.16±1.12	5.01±1.25	5.03±1.24	5.12±1.36	5.16±1.67	5.17±1.68

Table 3: Showing study parameters of group 10, group 11, group 12

S. No	Grp 10	Grp 11	Grp 12
SOD (U/ ml)	978.10±310.11**	1012.12±375.10	1014.13±270.09**
MDA (mole/gm)	210±20.14	221.08±22.09	220.10±21.07
GSH (mg/dl)	42.20±1.09	45.10±1.20	46.02±1.09
GPx (μ mole/GSH utilized)	73.01±0.82*	40.22±1.08	42.91±1.10
Vit E (IU/ mole plasma)	10.11±1.2	12.08±1.70*	14.07±1.8
Uric acid (mg/dl)	3.92±1.25	5.05±1.06	6.17±1.40

Table 4: Study showing parameters of group 1, group 2, group 7 and group 13

S. No	Group 1 (n= 6)	Group 2 (n= 6)	Group7	Group 13
SOD (U/ ml)	969.15±388.56	2840±201.60	1012.12±275.08	1002.11±215
MDA (mole/gm/min)	151±13.01	210±13.26	221.02±21.10	188.01±09.8*
GSH (mg/dl)	40.41±0.12	24.25±0.96	46.02±1.14	42.04±0.22**
GPx (μmole GSH utilized)	54.00±1.20	13.23±1.23	45.03±1.16	49.09±9.02
Vitamin E (IU/ mole plasma)	15.00± 1.04	8.00±1.40*	12.05±1.68	12.11±2.01
Uric acid (mg/dl)	4.56± 1.07	3.34±1.80	5.12±1.36	4.01±0.07

4. Discussion

Hypomelanosis is referred to as the decreased level of melanin in the epidermis, which results in two different kinds of changes (a) Decreased number or absence of melanocytes in the epidermis results in little or no melanin production (melanocytopenic hypomelanosis) e.g. vitiligo and (b) No decrease in the number of melanocytes but decreased melanin production. During the senescence process, the density of melanocytes in the skin decreases physiologically near about 10% per decade (Gilchrest., 1979)[8] but the loss of pigmentation can occur at any stage after exposure to myelotoxic agents.

Hypomelanosis can occur post-inflammatory and results from increased keratinocytes turnover that interferes with the melanosomal transfer as well as the activation of inhibitory cytokines. Neurochemical mediators such as norepinephrine and acetylcholine are toxic to melanocytes. Studies on vitiligo patients showed a higher level of plasma, urine catecholamine, and their metabolites, especially at the onset of the disorder (Cuchi et al., 2003).[9] A high concentration of norepinephrine and its metabolites may be due to a reduction in phenylethanolamine – N-methyl

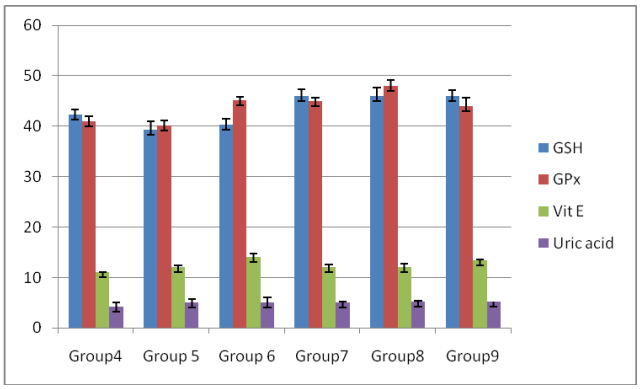

Fig. 4: Showing graph of GSH, GPx, vitamin E and uric acid in groups 4 to 9

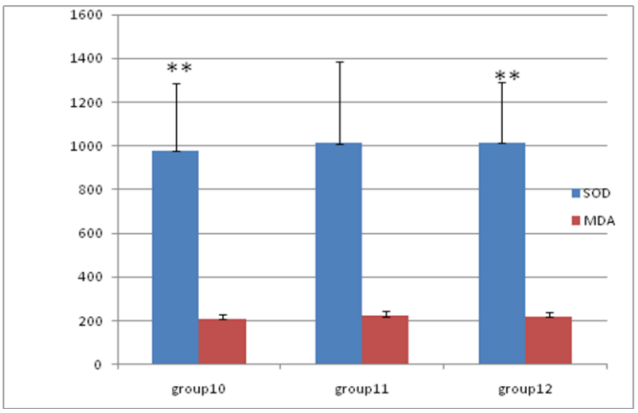

Fig. 5: Showing graph of SOD and MDA in groups 10 tp 12

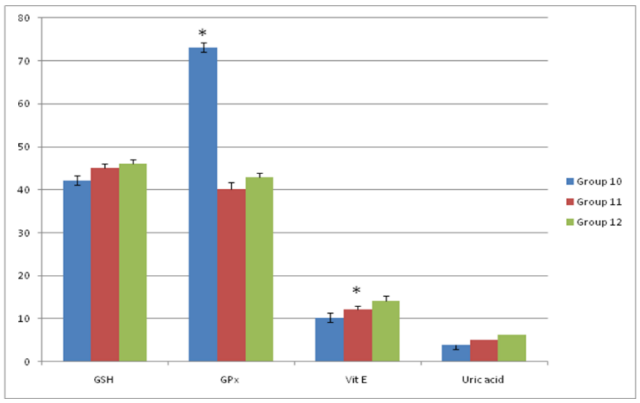

Fig. 6: Showing graph of GSH, GPx, vitamin E and uric acid in groups 10 to 12

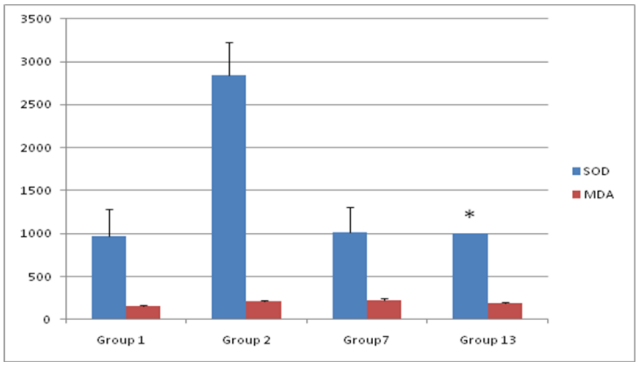

Fig. 7: Showing graphs of SOD, MDA in groups 1, 2, 7 and 13

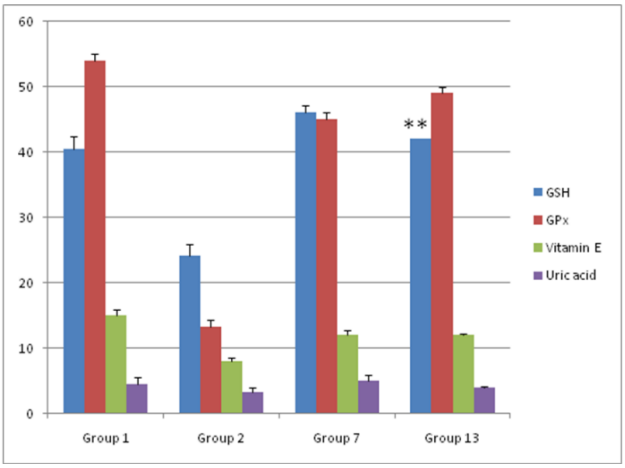

Fig. 8: Showing graphs of GSH, GPx, vitamin E and Uric acid

transferase (PNMT) activity and an increase in tyrosine hydroxylase (TH) activity. These enzymes convert L-tyrosine to L-dopa. Defective recycling of 6BH4 lead to the increased non-enzymatic production of 7BH4, concomitant with increased production of H2O2. The presence of 7BH4 in the epidermis initiates the process of depigmentation in vitiligo patients by blocking the L-Tyrosine supply to melanocytes. These alterations cause melanocyte destruction in vitiligo (Schalleuter et al., 1994).[10]

Tyrosinase is a key enzyme required for melanin synthesis. Tyrosinase catalyzes the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA), which is the rate-limiting step of melanin synthesis (Hearing VJ., 1999).[7] DOPA undergoes oxidation of dopaquinone, which is immediately converted to DOPAchrome and then to 5,6 di-hydroxy indole (DHI). Tyrosine-related protein (TRP2) converts dopachrome to dihydroxyindole carboxylic acid (DHICA). DHI and DHICA further polymerize to form eumelanin. Cystine/ glutathione reacts with dopaquinone to produce cysteinyldopas that undergo further cyclization to benzothiazines and higher condensates give rise to

photoprotection to the skin from ionization radiations (Krishnamurthy., 1969).[11]

It is not just a dermatological problem. It is a disorder of melanocytes. Melanocytes arise from the neural crest in embryonic life. The embryonic ectoderm also originates from the margins of the neural plate which forms the tubular central nervous system.

In the present study, we selected the plant Babchi belongs to the family Fabaceae described under the botanical name Psoralea corylifolia, described as Kalamashim in Sanskrit. Psoralea corylifolia Linn. is an annual herb growing throughout India. The plant is of immense biological importance and it has been widely exploited for ages, for its magical effect against several skin diseases like psoriasis, leucoderma, and leprosy. It is reported to contain essential oils, coumarins, alkaloids, flavonoids, and terpenoids (Krishnamurthy., 1969),[11] Ayurvedic Pharmacopoeia of India (1989). The active compound of Psoralea corylifolia Linn. is a psoralen.

Psoralen has been found to intercalate into DNA, where they form mono and di-adduct in the presence of long-wavelength UV light and thus are used for the treatment of hypopigmented lesions of the skin, like leucoderma (Vaidya., 2006).[12] The studied plant Psoralea corylifolia Linn. also contains oils, coumarins, alkaloids, flavonoids, and terpenoids. Major active components were bakuchiol, bakuchiol, psoralen, 7- methoxy bavachin, psoralester, and psorachrome 2.

In this study, we applied toxicant TBC (tertiary butyl catechol) 5% and 10% w/v in acetone, topically on shaved area of mice skin, which causes oxidative stress. Due to an increase in oxidative stress, the level of SOD increased in both groups (groups 2 and 3) in comparison to control.

Oxidative stress arises, and ROS are generated. The ROS non-competitively bind to the meta-phenolic position of tyrosinase, on meta-position electron density, decreases, and the structure of tyrosinase disintegrates. Groups 4 to 9 showed increased SOD levels. In groups, 10, 11, and 12 Psoralea corylifolia Linn seed oil was applied topically for 3 weeks, 5 weeks, and 7 weeks respectively. Group 12 showed a lowering in increased SOD levels in comparison to control. This group contains Psoralea corylifoia Linn. seed oil topically applied for 5 weeks. Group 12 was selected for further studies. Group 7 showed better results in comparison to the control and was combined studied with group 11 and better results were gained. This reveals that there is an imbalance in the oxidant-antioxidant system. Oxidative stress due to disease activity results in a high level of superoxide dismutase (SOD).

In vitiligo patients, the epidermal levels of ubiquinol vitamin E, reduced glutathione (GSH). An imbalance of the intracellular redox status and a significant depletion of enzymatic and non-enzymatic antioxidants are seen in the epidermis of vitiligo patients and represent the figure print of abnormal oxidative stress leading to epidermal cell injury (Passi et al., 1998).[13] A decreased GSH is associated with a strong decrease in tyrosine hydroxylase activity and melanin production in the skin (Bentham et al., 1999).[14] In groups 2 and 3 (toxicant groups) level of GSH decreases considerably and lead to the appearance of hypopigmentation in shaved area of mice. While therapeutic groups are showing an increase in GSH levels.

Alpha-tocopherol deficiency authenticates the susceptibility of the cell to oxidative membrane injury (Goth J., 2004)[15] which results in epidermal oxidative stress and may cause premature melanocyte death. In groups 2 and 3 levels of Vitamin E are decreased as compared to control, this promotes oxidative membrane injury. This decreased level of alpha-tocopherol was increased in group 7, group 11, and group 13. Whereas group 12 showed a relatively increased level of Vitamin E, so not chosen as good doses against vitiligo.

Reactive oxygen species (ROS) are capable of bleaching constitutional melanin and causing membrane lysis through lipid peroxidation reactions. The level of MDA was increased intoxicant group as compared to the control. Only group 11 showed a lowering in the level of MDA and all the other groups showed higher values of MDA, but not more significant than SOD and Vitamin E. Increased values of MDA cause membrane lysis, and the lowered values gained in two groups 11, helped to conclude better doses due to less cell membrane injury. As the cell membrane is less injured, the regeneration of melanocytes takes place and repigmentation appears.

Uricase convert uric acid to allantoin and H2O2. This H2O2 causes cell membrane injury and leads to melanocyte death. The level of uric acid decreases intoxicant group as compared to the control. Only group 11 showed an elevated level of uric acid as compared to toxicant groups 2 and 3. Group 12 showed more increase, but not significant, in the value of uric acid as compared to the control.

The impaired redox status theory states that in vitiligo melanocytes death results from an intrinsic increased sensitivity to oxidative stress that arises either from toxic intermediates of melanin precursors or from other sources (Njoo et al., 1998).[16] Low GPx activity leads to epidermal accumulation of H2O2 has been demonstrated in lesional and non-lesional skin (Gilchrest., 1979).[8] In toxicant groups 2 and 3 levels of GPx were decreased. The level of GPx was increased near normal in groups 11 and 13. Rest groups were not showing any significant change in GPx level as compared to the control.

Death of melanocytes is not essential for depigmentation in vitiligo but speculates either a primary effect on inhibition of melanogenesis or on the disappearance of melanocytes because of defective adhesion (Mosher et al., 1993).[17] First, the presence of residual melanocytes in the follicular reservoir gives rise to the repigmentation of vitiliginous

patches after phototherapy. Persistent melanocytes, which showed an ectopic distribution of pre-melanosomes within keratinocytes of the suprabasal layers, have been observed in lesional skin of long-standing vitiligo (Alkoy M et al., 2002).[18]

It has been proposed that vitiligo is a sequential two-stage disorder (Alkoy M et al., 2002.[18] In the first stage, tyrosinase activity decrease while in the second stage inhibition of melanization induces the death of melanocytes (Njoo et al., 1998).[16] An increased level of tension has been detected in the basal membraneand papillary dermis (Schalleuter et al., 1994)[10] suggesting that this extracellular matrix molecule inhibits the adhesion of melanocytes to fibronectin and thus might contribute to the loss of melanocytes in the vitiligo.

5. Conclusion

A present study was carried out to find antioxidant levels in depigmented skin samples and its neutralization by Psoraleya corylifolia Linn. therapy. An imbalance in the antioxidant system and free radical-mediated damage are initial pathogenic events in melanocyte degeneration in vitiligo.

The study was carried out on 78 C57/B6 mice. They were divided into control, toxicant a, toxicant b, ethanolic extract 100 mg/ kg b.w, 200 mg/kg b.w, 300 mg/kg b.w, petroleum ether extract 100 mg/ kg b.w, 200 mg/kg b.w, 300 mg/kg b.w orally, seed oil topically applied for 3 weeks, 5 weeks and 7 weeks and prophylactic study divided into 3 groups to check the best combination of oral and topical therapy best dose of aqueous extract 200 mg/kg b.w and seed oil topically applied for 3 weeks, 5 weeks and 7 weeks, having 6 animals each.

Where 100 mg/kg b.w of petroleum ether showed better results for SOD and MDA, while for GSH and vitamin E ethanolic extract 300 mg/kg b.w showed better results in comparison to the earlier one.

Due to the appearance of several adverse effects and increasing withdrawal of the patients from existing treatment nowadays, we designed this study, by concentrating on the antioxidant free radical scavenging concept, to minimize the adverse effect of existing therapy. The existing therapy includes 15 to 20 minutes exposure of to UVA and UVB every day, which causes psoriasis, inflammation, and hyperpigmentation side effects in patients. While in this study UVA and UVB exposure is avoided and the results are obtained. No psoriasis, inflammation, or hyperpigmentation was seen in the subject during the study.

This study is an Endeavour to take potent seed extract and seed oil of Psoralea carlifolia Linn. (described in Drayvagun by Acharya Priyavatt Sharma) for the treatment of vitiligo, which can be the most trusted and least harmful treatment for vitiligo, nullifying all the side effects of chemical drugs available and cheaper than the treatments available.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

References

1. Ortonne JP, Bose SK. Vitiligo: Where do we stand? *Pigment Cell Res*. 1993;8(2):61–72.
2. Bergqvist C, Ezzedine K. Vitiligo: A review. *Dermatology*. 2020;236(6):571–92.
3. Gupta D, Kumari R, Thappa DM. Depigmentation therapies in vitiligo. *Indian J Dermatol Venereol Leprol*. 2012;78(1):49–58.
4. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res*. 2003;2(2):90–100.
5. Elbuluk N, Ezzedine K. Quality of Life, Burden of Disease, Co-morbidities, and Systemic Effects in Vitiligo Patients. *Dermatol Clin*. 2017;35(2):117–28.
6. Wu JH, Cohen BA. The stigma of skin disease. *Curr Opin Pediatr*. 2019;31(4):509–14.
7. Hearing VJ. Biochemical control of melanogenesis and melanosomal organization. *J Investig Dermatol Symp Proc*. 1999;4(1):24–8.
8. Gilchrest BA. Effect of aging and chronic sunexposure on melanocytes in human skin. *J Invest Dermatol*. 1979;73(2):141–3.
9. Cucchi ML, Frattini P, Santagostino G, Preda S, Orecchia G. Catecholamines increase in the urine of non segmental vitiligo especially during its active phase. *Pigment Cell Res*. 2003;16(2):111–6.
10. Schallreuter KU, Wood JM, Ziegler I, Lemke KR, Pittelkow MR, Lindsey NJ, et al. Defective tetrahydrobiopterin and catecholamine biosynthesis in the depigmentation disorder vitiligo. *Biochim Biophys Acta*. 1994;1226(2):181–92.
11. Krishnamurthi A. The Wealth of India: Raw Materials; 1969.
12. Vaidya ADB. Reverse Pharmacological correlates of Ayurvedic drug actions. *Indian J Pharmacol*. 2006;38(5):311–5.
13. Passi S, Grandinetti M, Maggio F, Lowe N. Epidermal oxidative stress in vitiligo. *Pigment Cell Res*. 1998;11(2):81–5.
14. Benathan M, Virador V, Furumura M, Kobyashi N, Panizzon RG, Hearing VJ. Co-regulation of melanin precursors and tyrosinase in human pigment cells: role of cysteine and glutathione. *Cell Mol Biol (Noisy-le-grand)*. 1999;45(7):981–90.
15. Goth I, Rass P, Pay A. Catalase enzyme mutation and their association with disease. *Mol Diagn*. 2004;8(3):141–9.
16. Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PM. Nonsurgical repigmentation therapies in vitiligo: Meta-analysis of the literature. *Arch Dermatol*. 1998;134(12):1532–40.
17. Mosher DB, Fitzpatrick TB, Hori Y, Ortonne JP. Disorders of melanocytes. In: Fitzpatrick TB, editor. Dermatology in general medicine. New York: McGraw-Hill; 1993. p. 903–95.
18. Alkoy M, Celik VK, Ozelik S, Polat M, Marufihah M, Atlay A, et al. The effects of vitamin E on the skin lipid per-oxidation and the clinical improvement in vitiligo patients treated with PUVA. *Eur J Dermatol*. 2002;12(1):24–6.

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