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Effect Of Topically Applied Melatonin And Their Combination With Minoxidil Solution For Enhancement Of Hair Growth In Male Mice

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ABSTRACT

Background: Hair loss is considered important dermatological condition happened in both sexes but more affected on male. Hair loss is complex situation correlated with cosmetics issues especially in women. The current regimes have numerous limitations and serous side effect, so should be searching more convenient therapy with less side effect and more efficacy. The recent studies illustrated the positive correlation of elevation the level of total antioxidant status with improvement hair regeneration in hair follicle. Also the current research revealed the significant correlation between reduction of lipid peroxidation markers like F2 isoprostane and enhancement of hair growth. Melatonin has indoleamine molecule with antioxidant and vasodilatation activities. It has a central role in improvement hair growth by acting as capturing of ROS and decreasing both DNA damage and apoptosis mechanism via antioxidant properties. Objective: This research aimed to investigate the hair regeneration effect of melatonin in reducing hair loss in mice model by serial photographs, histological examination and measuring of peroxidation and oxidative stress markers (F2 isoprostane, total antioxidant activity) by Elisa assay in animal model and assess the efficacy for them. Materials and Method: In this study, 20 adult Wister Albino mice were used with 6-7 weeks in age and weighing 25-35 g. Coat hairs on the dorsal skin of male mice were gently clipped by electrical machine and then stained by using commercial dye. The adult mice were divided randomly into equal 4 groups (5 mice in each group) as the following:

1. Ethanol group: Considered as a control group, All 5 mice treated with the vehicle solution absolute ethanol alcohol, 0.3 ml applied by a micropipette to the uncover skin then spread by means of swab once daily for 3 weeks. Then skin tissue was collected after 3 weeks of study.
2. Minoxidil treated group: All 5 mice treated with Minoxidil solution 5%, 0.3 ml was applied to the denuded skin by a micropipette once daily for 3 weeks for. Then skin tissue was collected after 3 weeks of study.
3. Melatonin treated group: All 5 mice treated with MT solution 0.1%, 0.3 ml was applied to the denuded skin by a micropipette once daily for 3 weeks. Then skin tissue was collected after 3 weeks of application.
4. Melatonin plus Minoxidil treated group: All 5 mice treated with equal amounts of MX solution 5% + MT 0.1%, 0.3 ml of the mixed solutions is applied by a micropipette once daily for 3 weeks for all 5 mice. Then skin tissue was collected after 3 weeks of application.

Results: At the end of this experimental study, we showed that the tissue level of F2-IsoPs in MX, MT and MT plus MX groups are significantly lowered than those in control (Ethanol) groups. Also we showed that the serum tissue level of TAC in MX, MT and MT plus MX groups is significantly higher than those of control (Ethanol) groups, but the MT and MT plus MX treated group are more significant than MX treated group in elevation of level TAC. On the other hand, we showed that the hair growth and enlargement of hair follicle in MX, MT and MT plus MX groups are significantly increased than those in control groups. Also we showed that the tissue level of is significantly higher than those of both control and vehicle groups. In histology examination, we revealed that the MT and MX are resulted insignificantly increase in number of hair follicle in comparison with control, however, the number of hair follicle significantly increased in group treated with combination of MT plus MX when compared to control group. Conclusion: From the overall results, we approved that the Melatonin is significantly reduce hair loss in adult male mice via their pleiotropic effects as anti-estrogenic, anti-oxidant and vasodilatation activities.

Keywords: Melatonin, hair loss, oxidative stress, Minoxidil, antioxidant activity, F2-isoprostanes & TAC.

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ABBREVIATIONS

MX (Melatonin), MX (Minoxidil),Reactive Oxygen Species(ROS),F_2-IsoPs(F2-isoprostanes), TAC (total anti-oxidant capacity), Telogen Effluvium (TE), Oxidative Stress (OS).

INTRODUCTION

Hair loss or baldness is defined as common dermatological condition occurred in male and female but male more affected. It is characterized by miniaturization, thinning and fallout of hair from follicle in different parts of the body like scalp and it resulted from psychological status as adverse squeal [1-2]. Mechanism of hair loss is including two points: first one is a shortage of anagen phase resulted from abnormal cycle

and rapidly passage of anagen to telogen through catagen. The hair follicle has both dermal and epithelial component that causes remodeling in fast manner and decrease length of hair, the shaft is lost and hair enters telogen phase in high number [3].Second point is included dermal papilla or hair matrix are reduced in size that it causes modification in hair diameter and aspect. Hormonal changes, genetic factor, medical condition, drug use, stimulatory, inhibitory conditions, stress and anxiety are important causes for hair loss [4-5]**.** In general the hair loss is either visible hairlessness that called alopecia or hair losing occurred in high number more than from normal hair losing that known as effluvium [6]. Hair loss types commonly seen are alopecia, Telogen effluvium, male pattern hair loss and female pattern hair loss. Almost head is involved. Alopecia is divided into scaring and non-scaring hair loss. It classified into two form localized and generalized. Hair loss is ranged in severity from small area to involve the whole body. Baldness is not usually resulted from scaring or inflammation [7]. The causes of alopecia are either hormonal change or stress condition. Hormonal and genetic factor are the main causes for MPHL, while the FPHL of unknown cause. TE is result from psychological or physical factors [8].

Telogen effluvium is hair loss resulted from abnormal hair growth cycle (anagen phase defect), drug used, endocrine disease and stress condition [9]. Hair loss is classified into acute and chronic pattern. It belongs to class of non-scaring alopecia [10]. Female are more affected than male in TE because hormonal change occurred in postpartum and this lead to shedding of hair. The age and races are involved in epidemiology of TE. The prevalence of TE is not known exactly [11] [12].The TE is classified into two class either acute (<6 months) or chronic (>6 months), that including the shedding process of hair higher than normal shedding. TE resulted from defect in growth cycle of hair that it characterized by rapidly converted anagen to telogen without complete duration of anagen phase and this mechanism is occurred under physiological stress [13]. TE is become tiny and is decreased in size. Hair follicle regeneration is happened in acute TE. The density of hair in frontal and central scalp is decreased that it resulted from diffuse of loss and increase of shedding [12]. Hair loss treatment of TE included non-medical treatment and medical treatment. Non-medical treatment including acute state usually not need treatment because TE considers self-limiting and hair regrowth return to normal after termination of acute phase spontaneously during three to six month. In addition removed of trigger agents terminate this condition. Patient learning and education about hair loss in TE is main role in management of acute TE. Trigger factors are included either decrease nutrition supplement or drug uses. In general hair regrowth return to full density and complete appearance not more than one year's [14].In chronic TE medical use of topical MX will establish. MX is potassium channels opener and it caused vasodilator of arteries. This dilation is lead to reach blood and nutrient to hair follicle. Finally it promoting blood cycle in hair and increase hair growth by anagen phase promoting and decrease the shedding of hair (telogen phase)[15]. MX has not a role in treatment acute state but uses only in chronic. The deficiency of both mineral and vitamins in TE can be treatment by nutrition supplement. The surgical treatment by hair transplantation is not needed in TE[16-17], [12]. Oxidative stress (OS)is resulted from two condition either increment of synthesis free radical or inability the defense mechanism of the body to eliminate the excess of free radical or reactive oxygen species[18] (Prie BE, et al., 2015). The accumulation of free radical in body leads to cause damage to protein, lipid, cell membrane and DNA. This damage is resulted from correlation between excess of highly reactive molecules and unpaired electron and final result is formation of ROS. All these effects are resulted from either internal (such as inflammation, diseases or metabolism) or external processes (irradiation, pollution, food, drugs) [19]. The damage of ROS can be prevented or reducing by using antioxidant like (SOD, catalase, glutathione peroxidase, vitamin E, vitamin C, GSH), that it considered repairing body mechanism against oxidative stress and detoxify of ROS [20]. The total antioxidant capacity (TAC) can be defined as biological marker and it employed for calculating the quantity of free radical removed from biological samples by an analysis solution. The principle for this assay is estimated of TAC depending on reduction the metal cupper from copper (II) to copper (I) [19]. The antioxidant molecules are divided into enzymatic and non-enzymatic agent. The role of these molecules are maintained normal balance between oxidizing and non-oxidizing agent, reducing number of ROS, prevent accumulation of lot number and prevent damage of cell structure during oxidative stress [21]. The hair is exposed to the damage by increase of oxidative stress which that results from imbalance between free radical and repairing mechanisms [22]. The beneficial effect of antioxidazing agent (endogenous neutralizing mechanism) is neutralized and prevents the toxic effect of ROS (oxidizing agent) [23]. Other studies are showing impaired of endogenously repairing system lead to damage in hair follicle by free radical accumulation in high level. In turn this damage lead to diminishing pigment of hair and continuously hair loss or temporarily. These effects can be reversed by using supplement containing antioxidant [24-26]. Unsal Savc et al are found the significant correlation between pathogenesis of TE and the increment oxidative stress by disturbing the balance of Thiol/Disulfide Homeostasis [27]. F2-isoprostanes (F2-IsoPs) are molecules same as prostaglandins structure and regarded the one of lipid peroxidation markers. It resulted as end product from biological process for oxidation of lipid in membrane which that elicited by increasing of free radical. The possible explanation of formation F2-IsoPs are produced by interaction free radical with arachidonic acid. This lead to lipid peroxidation (arachidonic esters of phospholipid) and finally disturbed to membrane. It regarded a trusty indicator of oxidative stress and also has a role in inflammation process [28]. R. Belli et al. are clarified the level of F2-IsoPs increased in basal cell carcinoma in skin and reduced the level of F2-IsoPs after administration of vitamin E in topical form. These outcomes are suggested the F2-IsoPs plays a role in triggering the inflammation process and raised oxidative stress process. Also antioxidant effect of topical vitamin E reduced oxidative damage in skin [29]. Sara et al found the level of F2-IsoPs increased in patient with cutaneous leishmaniasis and this produced from disruption of balance oxidative and antioxidant. This study regarded the increase F2-IsoPs level trusty index of oxidative stress state [30]. Melatonin (N-acetyl-5-methoxytryptamine) is hormone produced in body of human and animal. It secreted from pineal gland and acts

outside of it. MT has many affected that it controls of body function. MT has highest peak in night because it synthesis mostly accomplished at night and low level at morning. The main effect of MT is antioxidant. This accomplished by scavenger of free radical and protect the cells from damage especially DNA. Because presented free radicals in high percentage leads to many unwanted effects and finally is resulted different diseases, so it conserved of the oxidant and antioxidant balance. Circadian rhythm (sleep-wake cycle) regulated by effect of MT that it regarded endogenous hormone. MT has indoleamine molecule in structure. It is synthesis from tryptophan precursor. Also it has vital role in regulated sleep, appetite, anxiety, immune response, cardiac function and mood disorder [31]. The mechanism action of MT is occurred through two types of intracellular membrane receptor that belonged to G-protein coupled receptor family. It works directly on two types of receptors melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2) [32][33]. Both of them when activated by melatonin binding that it resulted in decrease formation of second messengers (cAMP, cGMP), production of protein kinase A, C and cAMP are decreased also [34]. All these effects are appeared the pharmacological activity of melatonin by MAP kinases activation that it accomplished by binding of receptors [35]. Vázquez J, et al., has found MT acting as antioxidant by removed free radical as scavenger and prevent oxidative stress that lead to different disease[36]. MT plays an important role in reducing hair loss of androgenic alopecia in women by increasing or inducing anagenrate [37].It has anti-estrogenic effect by acting as estrogen receptor antagonist [38]. Ibraheem M, et al., found that hair growth was enhanced by topical application of MT for alopecia in Cashmere goat [39].Chun-He et al found topical administration of MT produced enlargement of hair follicle in Cashmere goat. The possible mechanism that explained the effect of MT on Cashmere goat resulted from the activity of MT in reducing the exposure of hair follicle to damage by oxidative stress (high level reactive oxygen species). MT is caused improvement the antioxidant enzymes efficacy [SOD and glutathione peroxidase (GPx)] and increased the level of total antioxidant capacity [40].

MATERIAL AND METHOD

Site and ethical consideration of the research

The study was done in the department of pharmacology and therapeutic and Middle Euphrates Unit for Cancer Researches, Faculty of Medicine \ University of Kufa. The study was accepted by Committee center of Bioethics in the University of Kufa and its representative in Faculty of Medicine. Whole procedures were done according to the recommendations of the Committee.

Animal grouping

In this study, 20 adult Wister Albino mice were used with 6-7 weeks in age and weighing 25-35 g was getting from the Center of Control and Pharmaceutical research/Ministry of health. Animals were harbored in animal house of Faculty of Science/ University of Kufa with a temperature controlled 20-25ºC and 60-65% humidity with a fitted 12 hrs light and 12 hrs dark cycles for 14 days before start of the procedures. In addition, the mice were freely access to food and water.

The hair mice covering the dorsal region were removed before experiment in one day by using diethyl ether as anesthetizing agent (inhalation route). The injury was avoided when hair shaving of mice in dorsal area by using electrical machine to shave the wanted region gently without damage. After cutting for hair by electrical shaving and removal all hair approximately, the surface layer of skin in this area appears with pink color. The indicator for resting phase of shaved area in mice was pink color [41].Then photographic with digital camera of each group after complete this shaving was performed (Figure 1).

Figure (1): Mice after clipping of the dorsal coat hair

The dorsal region of skin mice was appeared pink color without hair after clipping or shaving. Hoffmann was using dye commercial known to stain dorsal region of mice, after that alcohol was using to wash the area. Benefit from staining of dorsal part in this study to distinguish between regrowth area and denuded area and also to know the ratio white (area hair regrowth) to black color (area have not hair)[42][43].Photographic done after complete the staining of each mouse in all groups (Figure 2).

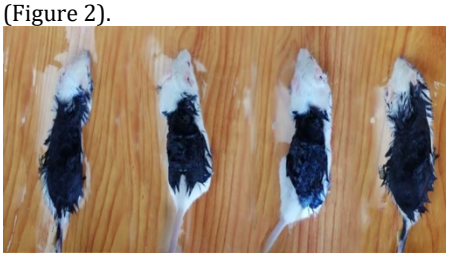

Figure (2-2): The mice after staining of the clipped area

In this study, the mice were divided randomly into 4 equal groups, 5 mice in each group and as the following:

1. **Ethanol group:** Considered as a control group, all 5 mice treated with the vehicle solution absolute ethanol alcohol, 0.3 ml applied by a micropipette to the uncover skin then spread by means of swab once daily for 3 weeks. After that, the skin tissue was collected.
2. **Minoxidil treated group:** All 5 mice treated with Minoxidil solution 5%, 0.3 ml was applied by a micropipette to the uncover skin once daily for 3 weeks. After that, the skin tissue was collected**.**
3. **Melatonin treated group:** All 5 mice treated with MT solution 0.1%, 0.3 ml was applied by a micropipette to the uncover skin once daily for 3 weeks. After that, the skin tissue was collected**.**
4. **Melatonin plus Minoxidil treated group:** All 5 mice treated with equal amounts of MX solution 5% + MT 0.1%, 0.3 ml of the mixed solutions is applied by a micropipette once daily for 3 weeks. After that, the skin tissue was collected**.**

Photographic Data Analysis

In this study we using Matlab 2015 data analysis program. This program was employed for inspection photographic data and special for this purpose. The white section represents hair growth part and black section represents hair naked part and was benefit from Matlab program to calculate ratio between them in dorsal region.

Preparation of the drug

The drug was prepared immediately before using by dissolved in absolute ethanol alcohols descripted by manufacturer (Medchemexpress).

Histological Section sanalysis

The histological sections were collected from samples at end period of study (after 3 weeks). This procedure was achieved by removing the hair from dorsal area by shaving and then was obtained 5mm of skin by excision. The small piece of skin was flattened shape, and then was using 10% buffered formalin to immerse the sample directly after excision[44](Castro JE, 1974).These sections were examined microscopically for the hair follicle number and diameter, in control and treated groups.

Assessment of tissue F_2-IsoPs and TAC

After experimental complete, the small parts of skin was immersing in cold PBS and putting in deep freeze (-80°C) for Eliza assay. Then the frozen skin portion was divided into small fragments and washed with cold PBS then the tissue was weighted and firstly homogenized by mortar and pestle with 1:10 (W/V) 0.1 M of precooled PBS (PH 7.4) contain 1% of protease inhibitor cocktail and 1% Triton 100X. For good homogenization, further breakdown the cell membranes achieved by subjected the homogenate to high intensity ultrasonic liquid processor. Lastly the homogenate was centrifuged at 14000 rpm for 15 min. at 4 ºC[45]. The supernatant was utilized to determine the level of F_2-IsoPs and TAC by ELISA kits.

Statistical analysis

Statistical analysis was done by using one way ANOVA test with post Hoc test at level of significance α= 0.05 to compare between control and treated groups, then performing multiple comparisons between the treated groups. Data were expressed as mean ± SD. Chi square test had been used to compare between the proportions of histological changes in various groups. Values of P< 0.05 were considered statistically significant. The statistical analysis had been done by using computer program SPSS version 26.

RESULTS

Effect of Melatonin on Hair Growth by Matlab Program

There was a significant hair growth (P value < 0.05) in the groups treated with MT, MX, and MT with MX as compared with control group (Table 1).

Table (1) Mean hair growth ratio by Matlab program of four experimental groups at the end of research (No of animals = 5 in each group) (Mean± SD)

Group	Mean± SD	P	Value
Ethanol	62.8±39.2	P	0.0001
MX	182±1.58	P1	0.0001
MT	142±1.6	P2	0.0001
		P3	0.001
MT plus MX	157.2±1.9	P4	0.0001
		P5	0.035

P: Total ANOVA, P1: Ethanol vs. MX, P2: Ethanol vs. MT, P3: MX vs. MT, P4Ethanol vs. MT plus MX, P5:MX vs. MT plus MX.

Effect of Melatonin and their combination with Minoxidil on the Number and Diameter of Hair Follicles by Histological Examination

The histological examination of the specimens showed no significant increase in the number of hair follicles (P value > 0.05) in all treatment groups except combination group (MT plus MX) that it was showed significant increase in number of hair follicle (Table 2).

Table (2): Mean the number of hair follicles by histological examination of four experimental groups at the end of research (No of animals = 5 in each group) (Mean± SD).

Group	Mean± SD	P	Value
Ethanol	79±16.05	P	0.026
MX	208±95.32	P1	0.191
MT	182.4±124.7	P2	0.292
		P3	0.792
MT plus MX	395±152.65	P4	0.003
		P5	0.062

P: Total ANOVA, P1: Ethanol vs. MX, P2: Ethanol vs. MT, P3: MX vs. MT, P4Ethanol vs. MT plus MX, P5: MX vs. MT plus MX. While hair follicle diameter has been increased significantly (P value < 0.05) in all treatment groups (Table 3, Figure 3, 4, 5, 6).

Table (3): Mean the diameter (in micrometer) of hair follicles by histological examination of four experimental groups at the end of research (No of animals = 5 in each group) (Mean± SD)

Group	Mean± SD	P	Value
Ethanol	0.3674±0.137954	P	0.034
MX	1.5994±0.34583	P1	0.002
MT	1.33900±0.656014	P2	0.01
		P3	0.467
MT plus MX	1.51260±0.633616	P4	0.003
		P5	0.807

P: Total ANOVA, P1: Ethanol vs. MX, P2: Ethanol vs. MT, P3: MX vs. MT, P4 Ethanol vs. MT plus MX, P5: MX vs. MT plus MX.

Effect of Melatonin and their combination with Minoxidilof TAC in Hair Follicles by Elisa

In our experimental research, we showed that the skin tissue level of TAC in treated groups (MX, MT, MT plus MX) was significantly (p < 0.05) higher than those level in control (Table 4).

Table (4): Mean TAC (in U) of hair follicles that measurement by ELIZA technic in examination of four experimental groups at the end of research (No of animals = 5 in each group) (Mean± SD)

Group	Mean± SD	P	Value
Ethanol	1.604±0.09	P	0.0001
MX	2.33±0.18	P1	0.0001
MT	4.488±0.11	P2	0.0001
		P3	0.0001
MT plus MX	8.234±0.0820	P4	0.0001
		P5	0.0001

P: Total ANOVA, P1: Ethanol vs. MX, P2: Ethanol vs. MT, P3: MX vs. MT, P4 Ethanol vs. MT plus MX, P5: MX vs. MT plus MX.

Melatonin decreased the lipid peroxidation marker in skin tissue (F2-IsoPs)

In this experimental study, we showed that the skin tissue level of F2-IsoPs in treated groups (MX, MT, MT plus MX) was significantly ($p < 0.05$) lower than those levels in control (Table 5).

Table (5): Mean F2-IsoPs (in nanogram) of hair follicles that measurement by ELIZA technic in examination of four experimental groups at the end of research (No of animals = 5 in each group) (Mean± SD).

Group	Mean± SD	P	Value
Ethanol	17.522±0.19	P	0.0001
MX	6.188±0.11	P1	0.0001
MT	15.298±0.19	P2	0.0001
		P3	0.0001
MT plus MX	9.198±0.18	P4	0.0001
		P5	0.0001

P: Total ANOVA, P1: Ethanol vs. MX, P2: Ethanol vs. MT, P3: MX vs. MT, P4 Ethanol vs. MT plus MX, P5: MX vs. MT plus MX.

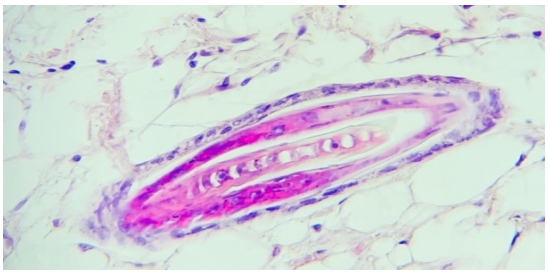

Figure (3): A cross section of skin mouse in control (Ethanol) group showing normal skin layers and hair follicles. H&E stain. (x400)

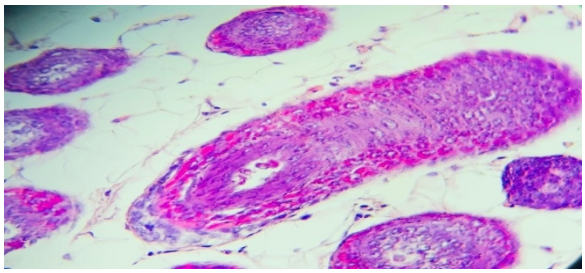

Figure (4): A cross section of skin mouse treated with MX showing normal skin layers, increase in hair follicle diameter. H&E stain (x400)

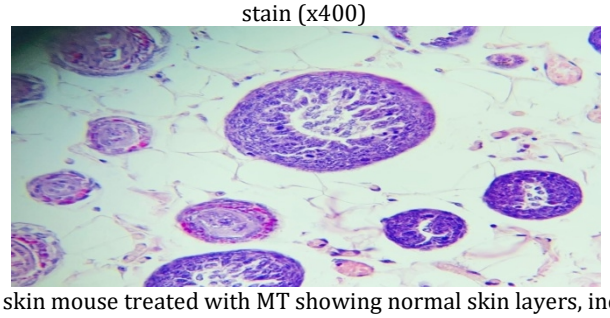

Figure (5): A cross section of skin mouse treated with MT showing normal skin layers, increase in hair follicle diameter. H&E stain (x400)

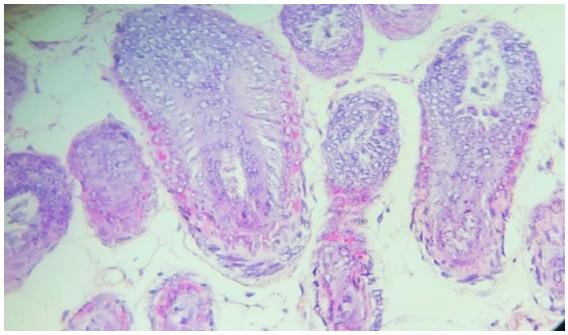

Figure (6): A cross section of skin mouse treated with MX plus MT showing normal skin layers, increase in hair follicle diameter. H&E stain (x400)

DISCUSSION

Hair loss or baldness is manifest by disappear of hair from any region of the head or body but especially frontal area of head. The severity of hair loss can vary from a small area to the entire body. The hair loss may be produced transitory or continuously disappearing of hair. Hair loss in some people produced from psychological issues. The hair loss is regarded a cosmetic issues especially in women and it considered a complex problem, so need to resolve this problem by increasing researches on hair loss to reach more conventional therapy to manage of this problem around world because the current regimes have unwanted adverse effect. MT is used for treatment insomnia and reducing high blood pressure by vasodilatation and antioxidant properties. These two mechanism can be clarified the possible effect of MT in increasing hair regrowth. So in our thesis, we estimated the hair growth effect of Melatonin, against control hair loss experimentally.

Effect of Melatonin on Parameters Study

Effect of Melatonin on hair growth

The experimental study confirmed that MT treated group produces a significant improvement in hair growth when compared with both groups (MX and Ethanol) after 21 days of treatment. However, this improvement became less than MX group. The study outcome is compatible with the result of Fischer 2004 that found the hair fall in women with androgenic alopecia decreased significantly when topical use of MT[37]. The study outcome is agreement with result of Fischer, et al., 2012 was elucidated significantly improvement of hair growth and elongation of anagen phase in AGA for both male and female. These effects were happened after using 0.1 % solution of MT in topical form [46]. Singh and Jadhav, 2014documentedthat MT has a significant role in improvement and adjust of hair regeneration, pigmentation and molting in numerous species like humans [47]. Fischer, et al., 2008, also was confirmed and getting same results [48]. The research of this study is agreement with outcome of Lorenzi S and Caputo R, 2003 illustrated significant decline in hair fall when using MT topically in treatment AGA patient for 6 months [49]. Although the result of this study is disagreement with outcome of Rimler A, et al., 2002thatMT has suppression effect on hair growth due to elicit nuclear exclusion of androgen receptor by MT [50]. In general MT has a significant role in promoting hair growth [46-49].The main explanation to increase hair growth after MT use due to induce and prolongation of anagen rate [37].

Effect of Melatonin on number of hair follicle

In The present study, the treatment with MT after 21 days is resulted insignificant increase in number of hair follicle when compared with MX and Ethanol group. Although this study outcome is disagreed with **Fischer, et al., 2012** was elucidated a significant increasing in hair count after treatment with MT in AGA patient for six month [46]. The main interpretation of elevation in hair count insignificantly may be returned to employ MT for short period.

Effect of Melatonin on diameter of hair follicle

In this study, MT significantly enlargement of hair follicle when compared with Ethanol group after 21 days of therapy, but it's not significant with MX group. The outcome of this study is agreement with Fischer, et al., 2012show MT employed of management of AGA men for six month was significant increasing in the size (diameter) of hair follicle, density of hair and texture significantly [46]. Our finding is agreement with Ibraheem, et al., and 1994which reported a significant correlation between enlargement of hair follicle in alopecia Cashmere goat and MT used [51]. The study done by Chun-He, et al., 2019 was illustrated that MT can be improving textile, quantity and quality of these fibers in Cashmere goat. Also it alleviated stress caused reduces of hair follicle regeneration [40]. Trueb RM, 2002discovered a significant correlation between the use of MT and increasing size of hair follicle and decline hair fall. Due to anti-androgenic properties of MT that it caused elongation of hair cycle [52]. The research of this study is agreed with study achieved by Peter Elsner and Meilen, 2001revealed that MT used in management AGA in women for six months cause significant enlargement of hair diameter for AGA patient[53]. The experimental study is agreement with study done by Brijesh kumar Patel, et al., 2017revealed that MT use produced significant expansion of hair bulb and enlargement in skin mice [54]. The possible mechanism for enlargement hair growth after MT use resulted from increasing MT2 receptor expression in late-anagen and catagen, and reduction activation in telogen and this lead to enter anagen hair bulb in active proliferated phase [48].

Effect of Melatonin on TAC in hair growth

Our study demonstrated that there was a significant elevation in level of TAC in MT treated group as compared with control group (Ethanol) and MX group. However, the elevation in TAC level was regarded more significant in MX. The research outcome is agree with Vázquez J, et al, 2017 that observed MT supplementation plays a significant role in suppression the excess ROS, decrease the level of GSH and potentiates defense mechanism against oxidative damage by acting as antioxidant properties [36]. Chun-He, et al., 2019 noted

MT plays a significant role in diminishing the oxidative stress that it happened in hair follicle of Cashmere goat. Topical application of MT produced increment of defense mechanism ability by potent antioxidant activity of it and elevation the level total antioxidant capacity [40]. This research is agreed with study accomplished by **Fischer, et al., 2006** elucidated a significant correlation between topically use of MT and reduction of oxidative stress and the cause for this decline return to metabolites of MT that it has same ability of MT in capture of free radical[55].The research outcome is acceptable with study achieved with **AL-Gaff AN, et al., 2005** revealed the use of MT in treatment AA patient(3 mg every other day for two months) resulted significant decline in hair fall by increasing total antioxidant capacity level [56]. MT caused control on hair growth cycle by increasing antioxidant capacity due to reduce ROS by working as scavenger of free radicals and this lead to increase DNA repair ,decline in apoptosis and control on hair growth cycle [48]**.**

Effect of Melatonin on F_2-IsoPs in hair growth

The present research has illustrated a significant lowering in level of F_2-IsoPs in MT treated group when compared with Ethanol and MX groups after 3 weeks of therapy. However, the lowering in level of F_2-IsoPs regarded more significant in MX group. The study was done by Lifu Zhang et al., 2006revealed a significant declined in F_2-IsoPs level after administration of MT (20 mg/kg) in rat with diquat-induced lipid peroxidation in vivo [57]. The outcome of this research is compatible with the study accomplished by Silvia Leoncini, et al., (2009),which reported a significant correlation between suppression of F_2-IsoPs in neonatal hypoxic-ischemic encephalopathy and administration of MT in injection form for seven days[58].AL-Gaff AN, et al., 2005 revealed a significant reduction in lipid peroxidation marker malondialdehyde (MDA) after using MT in AA patient (3 mg every other day for two months)[56]. The possible explanation of this mechanism produced from reducing lipid peroxidation and suppression of oxidative damage by Indole antioxidant activity of MT [57].

Effect of Melatonin plus Minoxidil on Parameters Study

The current study validated that combination groups of MT and MX produces a significant increasing of hair growth, enlargement of hair follicle, raising the level of TAC marker and reduction the level of F_2-IsoPs after three week of therapy when compared with Ethanol and MX groups. But this combination produced insignificant increment in number of hair follicle as compared with MX group, however this combination results a significant elevation of hair count as compared with Ethanol group. There are no instructive studies of combination therapy (e.g. MT plus MX). Our experimental study is regarded the first one that documented that this combination can act on promoting hair growth. The possible mechanism explained the action of combination drug MT and MX is not clear.

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