Annals of R.S.C.B., ISSN:1583-6258, Vol.25, Issue 4, 2021, Pages. 18703-18710
Received 05 March 2021; Accepted 01 April 2021

MEBO Ointment Acts As Antioxidant in Burns Patients

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ABSTRACT

Burn injuries are associated with anatomically within physiologically infections and biochemically within immunologically defect in hepatic functions during direct action on locally and systematic role of blood elements and liver enzymes. These defects represented the cellular response due to burn injuries. Aim of study: to assess the treatment and antioxidant action of MEBO ointment in medical therapy ,such as burn therapy. Methods: The study was performed by 66 burn patents(male) received treatment with ointment MEBO(100g)twice daily for two weeks and all patients have thickness burn injury, age ranged(15-65years for all subjects) and 50 men healthy control subjects ,So one day post burn samples were collected for hematological &biochemical parameters in kirkuk city at Azaadi hospital ,and this study done from march to December 2019 . Results: Leukocytes count (WBC), Neutrophil, lymphocyte,) and erythrocyte sedimentation rate (ESR) were increased, while red blood cells count (RBC), hemoglobin (HB) were decreased after burn injury. Creatinine and potassium element are increased significantly, while Sodium element was decreased and significantly increased in the liver enzyme and glutathione after treatment when MDA significantly decreased .Conclusion: This study concluded that blood parameters and liver functions were altered due to burn injuries and these modulations attributable to the lipid peroxidation(MDA), while the liver enzyme signaling of hepatic activity for glutathione synthesis for curbing the free radicals after treatment by MEBO ointment ,this productive mechanism may be new defect liver mechanism for burn injury.

Keywords:
burn, MEBO, GSH, MDA, Hepatic enzyme, blood parameters.

Introduction

Burn shock is the early complications of deep extensive stages of burns that constitutes the most causes of locally management in the burn patients and remains challenged ;therefore, the systemic treatments are not correct with timely[1]. The shock dominator by turbulence in membrane permeability for understanding of the pathophysiological state of accompanied during edema with exudation and evaporation within plasma losing and leads to viscosity increased of all hematological consequences during comparing with other forms of injury[2]. some burns can be associated with liver inflammations and tissue damaged may be lead to hepatic cell damage and reduced immune response due to apoptosis induction and necrosis may occurring in liver [3].The significant increase in the glutathione (GSH) is physiological condition widely distributed through internal organs and to the T- lymphocyte within liver cells and muscle cells , maybe producing abundant GSH for inducing some kind of inflammatory response and lipid peroxidation during the free radicals attacked with membrane and lead to loss of membrane permeability and cell damaged[4]. In the normal conditions of the body ,the cells have antioxidant defense mechanism of enzymatic actions which lead to attack the reactive oxygen species ROS for inducing the lipid peroxidation MDA, and this state lead to stimulated the activation of hepatic enzyme serum aspartate aminotransferase (AST),and alanine aminotransferase(ALT), which increasing after burn injury has been needed for some weeks to becoming in the natural stated after liver damaged[5]. Burn injury of liver lead to decrease substance synthesis by hepatic cell ,such as vitamin E and glutathione (GSH) with superoxide dismutase (SOD) and catalase (CAT) ,and those represented the first line defense mechanism of oxidative stress [6]. The liver plays a very important role in survival and recovery of burn patients through has been inflammatory response of metabolic process and immune functions

[7],but pharmacological treatment of burns enhance the antioxidant GSH activity and endogenous antioxidant defense mechanism for deleterious the effect of free radicals that appear to be fateful that suggested in some study[8] ,but the trace elements play important role in oxidative stress by formation free radicals ,such as zinc has ability to protect the cell from effects of oxidative damaged during reduced formation ROS(Reactive oxygen species) and hydroxyl radicals from peroxyl and superoxide radicals and formation (GSH),so strong treatment of burns lead to reduction of MDA and increased level of GSH is natural intracellular antioxidant [9]. MEBO is an oil based naturally used for treatment in Iraq country, containing berbeerine moisture and sitosterol promotes for wound healing by powering microbes a wide range , such as fungi [10] .The delivery of systemically applied antibiotics is impaired by the obstruction of blood vessels in burn injury, so the medicated by MEBO ointment are leading to penetrates the membrane and enhanced the activity of pseudomonas and losses the pain[11],due to is consist from pure herbal and natural origin and a Beeswax with sesame oil, which has ability to reduce severe pain of burns during debars the shock due to reducing the dermal injury and enhance the activity of phagocytic cell such as granulocyte and monocyte has been promotes some consequences substance for resistance the burn injury during inhibition bacterial infections ;therefore increased WBC and platelet count for coagulation substances that reduction the erythropoiesis and leukocytosis was evinced and steady in the advocacy state[12].

Patients &methods

This study was performed on 66 burn male patients, their ages range from 15 to 65 years and divided in two group according to age ranged, each groups included 33burn patients, with different degrees of burn injury ,and the study done period from March to the December 2019. All were treated by Moist Exposed Burn Ointment (MEBO) according to hospital program, 1% MEBO ointment was applied on the affected area for burn treatment as a local injury twice daily. In patients with duality burned body and was managed for separating either in group 1 and group 2 of burn patients and used 100g for treatment .The study were included 50 healthy control male subjects. Ten milliliters Blood sample were collected by venipuncture from all subjects after 48h –post of burn, and at 2^{nd}, 7^{th}, and 12^{th},day to check different parameters including blood picture, serum creatinine and hepatic enzymes. The patients divided in two groups depended on their ages ; group one from 15-35 years old (young patients) and group two from 36- 66 years. Old (old patients) after recording their burn history .Measuring MDA estimated by formation of thiobarbituric acid reaction with TBA-RS of tissue with ice-cold 150nMkcl as described by.[13] using $1.56x\ 10^5 M^{-1} cm$.GSH was measured by modified of Eliman producer and centrifuge 1077xg for 5 min and 0.5 ml of serum was putting of o.3 ml/l Na2HPO4 2H2O for measuring GSH level [14]. Potassium measuring by used flame spectrometer for measuring potassium concentration in the serum during pass only light violet from monochromatic filter for potassium at 767 nm absorbance intensity and sample was diluted with ratio of 1:100 with ion-free water and same method was performed for measuring sodium in serum by passing the yellow light in the model is at a wavelenghth of 589 nm,and the sample diluted at 1:100 [15].Hepatic enzyme activity were measured, by Enzyme assay fluorescence (DU640 UV/Vis Beckman coulter)[16].

The statistical test was doing by ANOVA test using the SPSS version 18. Results were reported as mean ± standard deviation .

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Results

Table, (1) shows the levels of MDA and GSH in burn and control subjects during the course of the study. Results show significantly (P<0.05) decreased in MDA after 2 days treatment with MEBO at 2 days and gradually decreased later on up to the 12th day to reach 12.7 mmol/ml, from 55.5 mmol/ml pretreatment after burn. On the reverse GSH, was gradually increased after treatment from 2.17 mmol/ml before treatment to 12.12 mmol/ml after 12 days, in younger group (15-35years old) and to 5.29 mmol/ml in older group (35-65years old).

Table(1) levels of MDA & GSH in burn patents pertreatment and post treatment with MEBO.

creteria	Pretreatment group(n=33)	Days after treatment(n=33)	Ages15-35 years group	Ages 35-65 years group
MDA(mmol/ml)	**7.18 ± 55.35**	Day 2second	15.59±1.3	16.3±1.2
		Day7 sixth	14.2±1.2	11.9 ±0.3
		Day12	12.7±1.3	11.6±0.7
GSH (mmol/ml)	**2.17 ± 66.89**	Day second 2	5.93±0.3	3.8±0.3
		Day7 sixth	9.21±0.2	4.21±0.4
		Day12	12.12±0.1	5.29±0.1

The level of hepatic enzymes were significantly (P< o.o5) increased after 6 days within treatment by MEBO ointment. AST, ALT and ALT, reach 850±33, 890 ±47, and 780 ±28 U/L, from 42 ±10, 57±10, 157 ± 19 U/L respectively. As for sodium and potassium, there was significant (P< 0.05) decrease in sodium from 138±5 mmol/L slight increase in potassium from 6.5±11 to 8.5±20. **show table2**

(Table2) level of hepatic enzymes &electrolyte in burn before and after treatment with MEBO.

Hepatic enzyme	Burn patient pretreatment N=33	Burn patient's after treatment (n=33)
AST U/L	42±50*	850±33
ALT U/L	57±10*	890±47
ALP U/L	157±19*	780±28
Na +(mmol/l)	138±50**	120.9±30
K +(mmol/l)	6.5±11**	8.5±20

The blood parameters and serum creatinine as compared between normal people, and burn patients. Results show significant decrease in total red blood corpuscles count from 5.7 in normal peoples to 4.14,4.30,and 4.4 million, in day2,6,11respectively in younger patients and 4.2, 4.28,and 4.53 million in day 2, 7, 12 in older patients after treatment with MEBO. same trends was found in hemoglobin levels which decreases from 13.23g/dl to 9.21g/dl in the 2nd day after burn, pretreatment in older patients. Results of Erythrocyte sedimentation rate, (ESR) were highly increased from19.9 mm/h to 60.6 mm/h in the second day after burn in young group and to

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59.4mm/h in older group. At day 6th start to decease in both groups to reach 44.8/h, and 45.6mm/h in young group and older age group respectively, at day 12th after burn by MEBO treatment This indicate an improvement in the way by MEBO. The increase in ESR attributed to fluid oozes to blood vessels, RBC hemolysis, and reduced RBC count.
Leucocytes count, and neutrophils, basophils, and lymphocytes percentage, showed significant increase, especially in old patients.
WBC count was5.1x 10^3 /mm^3 in normal peoples increased to 16 1x 10^3 /mm^3 after burn in young patients and to 14 1x 10^3 /mm^3 in old patients.
Neutrophils, basophiles, and lymphocytes percentage were increased from normal range (49.9%, 0.7%, and 21.9% for neutrophils, basophiles, and lymphocytes, respectively) to maximum increase (66.0%,1.85%, and 38.4% for neutrophils, basophiles, and lymphocytes, respectively).**show table3**
Results also showed significant increase in serum creatinine levels in burn patients from 82.8±4.81mmol/l in control groups ,but 148.5±12. 6mmol/l in young patients after 12 days, and129.24±21.18mmol/l in old patients after 12 days, respectively. These increases were persist up to the 11th day after treatment.

Table3 level of blood elements & creatinine in burn patents

Blood parameters	Normal healthy Control N=50	Group 15-35 years old N=33 patient			Group 36-66 years old N=33 patient		
Days after treatment		Day2	Day7	Day12	Day2	Day7	Day12
RBC count x^6/mm	5.72 ±0.70	4.14 ±0.42	4.30 ±0.53	4.4 ±0.65	4.02 ±0.57	4.28 ±0.57	4.53 ±0.55
HB g/dl	13.23 ±0.8	10.20 ±3.15	10.2 ±1.31	10.7 3±3.55	9.21 ±0.716	10.17 ±0.74	11.2 ±1.32
ESR mm/hour	19.6 ±1.51	60.6 ±1.71	57.0 ±2.80	44.8 ±6.55	59.4 ±2.20	49.9 ±7.89	45.6 ±6.45
WBC COUNT $X10^3$ / mm^3	5±.22 ±6.64	16.97 ±1.65	15.36 ±1.02	15.36 ±10.27	14.20 ±0.72	14.46 ±0.63	13.73 ±3.94
Neutrophils%	49.9 ±5.53	71.0 ±3.46	69.4 ±4.30	66.0 ±3.24	66.8 ±22.60	66.8 ±2.86	67.0 ±2.58
Basophiles %	0.70 ±0.67	0.95 ±0.80	1.35 ±0.47	1.30 ±0.42	1.25 ± 0.35	1.85 ±0.34	1.61 ±0.50
Lymphocytes %	21.90 ±2.33	28.80 ±2.57	36.23 ±4.37	31.30 ±2.98	38.40 ±2.33	29.20 ±3.79	29.0 ±2.54
Creatinine mmol/l	82.8 ±4.81	87.10 ± 3.50	95.8 ±2.67	148.5 ±12. 6	96.4 ±1.26	178.40 ± 20.39	129.24 ±21.18

Discussion

After the remedied by MEBO lead to highly progressive reduction of edema and clinical inflammation was harmless ;therefore, the observation of the MEBO ointment and application contributed to the debridement of the wound facilitating and rapid epithelialization within 4 to 7

days and pain scores was decreased gradually after treatment, during reduction MDA and this result agree with[17]. After following assessments at days 7 to 12 and 14 the changes in the pain scores become less than due to the first day ,and this result agree with[18]. Although burn injuries regarded as sterile injuries yet pathophysiological changes could be occurs not only in the location but also internal organs and tissues during liver damage and hyper-permeability response increased ,which is suggested by[19]. Early onset of liver dysfunction were found in response to the burn injuries acquiring elevated reactive oxygen species and these changes were founding in all 66 patients regardless the age, which is suggested by[20] . MDA as a markers for peroxidation were increased after burn, and significantly reduced after treatment with MEBO (table1)and this result agree with[21]during mention an increased level of formation of reactive oxygen species as indicated by MDA ,some study suggested that ,the GSH is probably recruited during burn injury to protect cellular and extracellular proteins from irreversible oxidations. [22] ,which is reported that ,the MDA levels were significantly higher in all degrees of burns ,And these physiologic changes lead to aggravated the whole body inflammatory response and bully cycle of accelerating organ dysfunction ,this result agree with[23]. The declines of MDA level significantly after treatment reveal that MEBO have an antioxidant activity ,and this result agree with [24], However, within cellular injury and changes in cell membrane permeability lead to the liver enzymes leaking to the circulation and increased their levels after burn injury ,which is reported by[25]The hepatic cells can be synthesis and secretion different types of proteins; such as bone marrow, which is promote further hempoetic responses and increasing white blood cells count ,so the levels of sodium was decreased and potassium was increased, may be defense response for preventing heart failure, which is suggested by [26],and these changes attributed to edema and hyper permeability .Treatment with MBEO start to restore normal values of liver enzyme after 7 days, similar results were reported earlier [27],and low sodium and high potassium level after 12 days of burn injury treatment and this result agree with [28].Blood parameters were significantly altered due to burn injury in both young and old patients perform to decrease RBC count from 5.72 million to 4.14 and 4.02 million in young and old patients respectively. Hemoglobin level, also decreased from 13.23 to 9.21mg/dl in old patients, and to10.2mg/dl in young patients; These results were attributed to bleeding and loose of tissue fluids with hemolysis of RBC especially in 2nd degree burns and start to restore at day12 after treatment with MBEO. Erythrocyte sedimentation rate,(ESR) were increased in burn patients as compared with normal peoples, this increased could be results from fluid ooziness to blood vessel with RBC distraction formation of hypoproteinemia ,and reported by [29]. Total leucocytes(WBC), and their differential count were significantly altered were there was an increased number of WBC count ,such as neutrophil and lymphocytes percentage as shown in table-3, these results reflect first defense mechanism in body during inflammation ,and suggested by [30].Basophiles percentage, also increased in burn patients due to allergic reactions and tissue destruction ;therefore burn injury stimulated mast cell to degranulation and caused more instantaneous secretion of histamine and cytokines by tissue inhabitant mast cells, which is suggested by [31],so the basophils and mast cells have long been known to play critical roles in allergic disease and host defense. Creatinine levels is an indicator of kidneys function changes according to burn condition and Indicated systemic effects of burns, especially kidneys functions ,and this result agree with [32], Creatinine levels indicated moderate change in the quantity within the normal range 74.3mmol/l;although there is significant increase in the creatinine level in the burn groups compared with control groups and may be attributed to minor effect of burn to kidneys ,and this result agree with[33] .

Conclusion

The study confirmed MEBO ointment improved dressing burn significantly at first 15 days compared with control groups and it is useful for early management of burn injury during increased neutrophil and GSH activation and decreased MDA which appear sources of oxidant in burns.

Acknowledgement

We would like to thank all persons who helped to carry out this study, particularly Kirkuk hospitals and Doctors.

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