IJP (2014), Vol. 1, Issue 3 **(Research Article)**

Received on 30 November 2013; received in revised form, 24 January 2014; accepted, 26 February 2014; published 01 March 2014

WOUND HEALING AND *IN-VIVO* ANTIOXIDANT ACTIVITY OF MIMUSOPS *ELENGI* LINN. LEAF EXTRACT

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Keywords:

Mimusops elengi, Wound healing, Betadine, Antioxidant, Alkaline phosphate

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ABSTRACT: Objective: Phytochemical and pharmacological investigation on the leaves of *Mimusops elengi* Linn. for wound healing and *in-vivo* antioxidant activity in albino mice. **Material and Methods:** The methanolic extract of leaves of *Mimusops elengi* was studied for its wound healing effect on excision wound model in mice and *in-vivo* antioxidant activity at the dose level using a biochemical estimation of blood serum in mice. **Result:** Preliminary phytochemical analysis revealed the presence of saponin, carbohydrate, glycosides, flavonoids, tannin and phenolic compounds. *Mimusops elengi* methanolic extract (MEME) was examined for wound healing activity in the form of ointment in excision wound model in mice. The extract ointment showed a considerable response in the above model as compared to standard drug Betadine ointment regarding wound contracting ability, wound closure time. Histological analysis was also consistent with the proposal that *Mimusops elengi* leaf extract exhibits significant wound healing effect. As antioxidant action has been reported to play a crucial role in the hepatoprotection in *in-vivo* model of antioxidant activity. It has been found that it reduces both serums ALP and Bilirubin. Treatment with CCl_4 increases the level of total lipid, total triglycerides and total cholesterol in the liver which was reduced on the administration of a methanolic extract of *Mimusops elengi*. **Conclusion:** The methanolic extract possesses wound healing and *in-vivo* antioxidant activity.

INTRODUCTION: *Mimusops elengi* Linn. (Sapotaceae) commonly known as Indian Medlar Tree, Bullet Wood, Spanish cherry (English), Molsari (Urdu), Bakul, Bolsari (Hindi), Pogada (Telugu) [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.1(3).200-06

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.1(3).200-06

It is a small to a large evergreen ornamental tree which is highly used in traditional medicine for the treatment of various diseases. It is distributed throughout South India, Burma, Pakistan and the Andaman Islands in evergreen forests and grown as avenue tree [2]. Various morphological parts of the plant are used as a remedy in various ailments in the indigenous system of medicine for centuries.

The extract of the flower is salutary not only in heart diseases but also used as antidiuretic agent in polyuria condition, expectorant and for asthma. It alleviates the toxins, hence used as an anti-toxin [3].

Floral part of the plant produces a copious discharge from nose; sniffing is employed to relieve a headache [1]. It is also used to prepare lotion for wounds and ulcers; dried powder is a brain tonic and is useful to relieve cephalalgia. Internally bark skin is benevolent in leucorrhoea, menorrhagia and is also known to have antiulcer activity [4, 5]. The bark is used as a tonic, febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums, anti-HIV Type 1 protease.

Unripe fruit is used as a masticatory and will help to fix loose teeth [6]. A seed bark decoction is used as an aphrodisiac, cardiotonic and to treat mouth ulcer. Latex is applied to treat scabies and skin sores [7]. Leaves are used as an antidote in snakebite [8]. The presence of saponins, alkaloid, steroids, and terpenoids has previously been reported from *M. elengi* [9]. The flower contains volatile oil [10], D-mannitol, beta-sitosterol and beta-sitosterol-D-glycoside [11]; leaves contain sterols, reducing sugar, tannins; stem bark contains tannins, spinosterol and taraxerol [12].

Some preliminary phytochemical investigations reported that flavonoids are present in leaves of the *Mimusops elengi*. Therefore, the objectives of the present study were to investigate the wound healing and *in-vivo* antioxidant activity of the methanolic extract of *Mimusops elengi* (MEME) leaves in mice. A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. According to the wound healing society, wounds are physical injuries that result in an opening or breaking of the skin that causes a disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue [13].

Classification of Wounds: Wounds are classified as open and closed wound on the underlying cause of wound creation and acute and chronic wounds by the physiology of wound healing.

Open Wounds: In this case, blood escapes the body and bleeding is visible. It is further classified as Incised wound, Laceration or tear wound, Abrasions or superficial wounds, Puncture wounds, Penetration wounds, and gunshot wounds.

Closed Wounds: In closed wounds, blood escapes the circulatory system but remains in the body. It includes contusion or bruises, hematomas or blood tumor, crush injury, *etc*.

Acute Wounds: An acute wound is a tissue injury that normally precedes through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time frame [14].

Chronic Wounds: Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation chronic wounds either require a prolonged time to heal or recur frequently. Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, malnutrition, immunodeficiency or medications are the most frequent causes of chronic wounds [15].

Mechanism of Wound Healing: The response to injury, either surgically or traumatically induced, is immediate and the damaged tissue or wound then passes through three phases to affect a final repair:

- The inflammatory phase
- The proliferative phase
- The remodeling phase

The inflammatory phase prepares the area for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted. The fibroblastic phase rebuilds the structure, and then the remodeling phase provides the final form.

Inflammatory Phase: The inflammatory phase starts immediately after the injury that usually last between 24 and 48 h and may persist for up to 2 weeks in some cases. This phase launches the hemostatic mechanisms to immediately stop blood loss from the wound site. Clinically recognizable cardinal sign of inflammation, rubor, calor, tumor, dolor, and function- laesa appear as a consequence. This phase is characterized by vasoconstriction and platelet aggregation to induce blood clotting and

subsequently vasodilatation and phagocytosis to produce inflammation at the wound site [16].

Proliferation Phase: The proliferation phase essentially involves the generation of the repair materials and the majority of the skeletal muscles injuries [17].

Remodeling Phase: The remodeling phase is an essential component of tissue repair and is often overlooked. The outcome of these combined events is that the damaged tissue will be repaired with scar [17]. Antioxidants are intimately involved in the prevention of cellular damage. The common pathway for cancer, aging, and a variety of diseases [19].

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. They are substances that protect cells from the damage caused by unstable molecules known as free radicals. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants [20].

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although several enzyme systems within the body scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category [20].

Liver diseases, especially viral hepatitis occurs predominantly in the developing world with an enormous impact on public health & economy. Carbon tetrachloride (CCl_4) is widely used in animal models to induce acute liver injury. It is generally believed that the toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome P450 enzyme system into a highly reactive free radical trichloromethyl radical. In the present study, an attempt has been taken to elucidate the effect of the extract of *Mimusops elengi* on CCl_4 induced hepatic damage concerning biochemical marker enzymes & histopathology. The result of this In vivo study may support the plant to be good a herbal antioxidant drugs.

MATERIALS AND METHODS:

Plant Material: *Mimusops elengi* Linn. leaves were collected from Jhansi (UP) during September 2012. The plant material was identified and authenticated by Dr. Neelima Sharma, National Vrkshayurveda Research Institute, Gwalior Road, Jhansi with reference no. 21246**.**

Drugs and Chemicals: All standard chemicals used in this study were of analytical grade. The drugs Betadine ointment was obtained from WIN Medicare Pvt. Ltd., Hyderabad and Silymarin from Lupin Pharmaceuticals, Indore.

Preparation of Extract: The Leaves of *Mimusops elengi* were air dried, and then these were made into coarsely powdered form. The powdered drug (about 150 gm) was packed in soxhlet apparatus and continuously extracted with methanol (25-30 ºC) till complete extraction, after complete extraction the solvent was concentrated under reduced pressure and controlled the temperature in a rotary evaporator until it becomes completely dry. The percentage yield of the leaf extract was 20.71 gm (13.80%).

Preliminary Phytochemical Screening: A preliminary phytochemical screening of the extracts revealed the presence of Saponin, carbohydrate, glycosides, flavonoids, tannin and phenolic compounds.

Animals: Healthy albino mice (40-50 gm) of either sex (bred in D.R.D.O Gwalior, M.P.) were used. The animals were obtained from animal house of the Institute of Pharmacy, Bundelkhand University, Jhansi; India. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal's house temperature was maintained at 23 ± 3.0 ºC with a 12 h light/dark cycle. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) of the Institute with reference no. BU/Pharm/IAEC/12/027 (approved by CPCSEA Regd No. 716/02/a/CPCSEA).

Acute Toxicity Study of the Extract: An acute toxicity study was performed for the extract on Healthy albino mice (40-50 gm) according to Organisation for Economic Co-operation and Development (OECD)-425 guidelines. The method of Up and Down was used to determine the dose. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally 100, 200, 500, 1500, 2000 mg/kg dose and percent mortality was observed after 24 h then 72 h and after that once daily for 14 days.

The Methanolic extract of Leaves of *Mimusops elengi* (Linn.) did not cause any mortality up to 2000 mg/kg and was considered as safe. The oral LD_{50} of the Methanolic extract estimated in mice must be 2000 mg/kg.

Wound Healing Activity:

Excision Wound Model: Excision Wound healing was used to evaluate the wound healing activity of the extract. Circular wounds were inflicted on the cleared skin by cutting under mild Xylocaine 4% topical anesthesia. The areas of the wounds were measured (sq. mm) immediately by using vernier calipers. This was taken as the initial wound area reading. The animals were divided into three groups of eight animals each Healthy albino mice.

Group I received Simple ointment base

Group II received Betadine ointment (10% w/w)

Group III received MEME (10% w/w)

The wound area of each animal was measured on 1^{st}, 4^{th}, 8^{th}, 12^{th}, 16^{th}, 18^{th} post wounding day. The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelialisation time that indicates the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialisation **Table 1**.

TABLE 1: EFFECT OF *MIMUSOPS ELENGI* EXTRACT ON EXCISION WOUND MODEL IN MICE

S. no.	Group	4^{th}	8^{th}	12^{th}	16^{th}	18^{th}	Epithelization Time
1.	Control	425.33 ± 1.33 (15.55%)	338 ± 1.15 (32.89%)	211.66 ± 1.2 (57.97%)	77.33 ± 1.76 (84.64%)	13.03 ± 1.23 (97.52%)	21.5 ± 0.34
2.	Betadine	390.12 ± 3.3 (22.69%)	280.6 ± 1.42** (44.38%)	105 ± 1.20** (62.58%)	9.66 ± 1.10 ** (98.08%)	5.740 ± 0.10 ** (98.86%)	19.4 ± 0.2**
3.	MEME (10% w/w)	366 ± 2.78** (27.61%)	268.6 ± 1.7 (46.9%)	125 ± 1.34** (75.25%)	7.34 ± 1.24** (98.54%)	4.85 ± 1.02** (99.04%)	17.16 ± 0.1666***

Values are mean ± SEM where ** P<0.01, *** P<0.001

Wound Contraction and Epithelialization Time: [21]

% of wound contraction = Wound area on day 0 − wound area × 100 / Wound area on day 0

Where n = number of days 4^{th}, 8^{th}, 12^{th}, 16^{th} and 18^{th} day.

Histopathological Studies: The healing tissues obtained on the 18^{th} day from all three groups of animals of the Excision Wound model were processed for histological study. Sections were qualitatively assessed under the light microscope and observed in respect of fibroblast proliferation, collagen formation, epithelialization and blood vessels.

Statistical Analysis: Treated group was compared with the control group. The results were analyzed statistically using Students t-test to identify the differences between the treated and control. The data were considered significant at $p<0.05$.

***In-vivo* Antioxidant Activity:**

Biochemical Estimation: Hepatopathy was induced in animals by administration of CCl_4 intraperitoneally (i.p).

The animals were divided into six groups of four animals each mouse.

Group I: received Normal control (9% NaCl)

Group II: received CCl_4 (1.25 ml/kg,i.p, in liquid paraffin/14 days)

Group III: received Standard drug Silymarin (100 mg/kg/14 days)

Group IV: received MEME (100 mg/kg/14 days) suspended in polyethylene glycol.

Group V: received MEME (200 mg/kg/14 days) suspended in polyethylene glycol.

Group VI: received MEME (500 mg/kg/14 days) suspended in polyethylene glycol.

At the end of the treatment, rats were sacrificed by cervical dislocation; blood samples were collected by collected by direct cardiac puncture and serum was used for the assay of marker enzymes and other parameters, the liver was dissected out and immediately preserved in 10% formaldehyde solution for histopathological study.

Serum Biochemical Estimations: Cholesterol, Total Protein, Total Bilirubin, Serum glutamate-oxaloacetate transaminase (S.G.O.T) or Aspartate aminotransferase (AST), Serum glutamate pyruvate transaminase (S.G.P.T) or L-alanine amino-transferase (ALT), Alkaline phosphate (ALP) were estimated using standard commercial kits from SPAN India Ltd. Surat, India [22].

TABLE 2: EFFECT OF *MIMUSOPS ELENGI* EXTRACT ON SERUM BIOCHEMICAL PARAMETERS IN MICE

S. no.	Group	Cholesterol	Total Protein	Total Bilirubin	SGOT	SGPT	SALP
1	Control	98.23 ± 3.12	7.58 ± 0.62	0.93 ± 0.2	38.42 ± 1.48	31.45 ± 1.63	66.21 ± 6.4
2	CCl_4	281.2 ± 3.30	4.94 ± 0.27	1.85 ± 0.25	111.3 ± 3.5	88.4 ± 2.5	80.26 ± 2.2
3	Silymarin	133.8 ± 1.92	7.70 ± 0.16	1.4 ± 0.3	41.02 ± 0.6	39.41 ± 1.72	69.11 ± 0.16
4	MEME-100mg/kg	148.4 ± 1.61	6.91 ± 0.18	1.6 ± 0.21	56.34 ± 2.03	52.43 ± 2.32	78.63 ± 2.19
5	MEME-200mg/kg	126.2 ± 2.40	7.73 ± 0.20	1.52 ± 0.3	48.3 ± 1.66	44.81 ± 3.07	78.28 ± 3.32
6	MEME-500mg/kg	100.1 ± 4.1	7.85 ± 0.41	1.2 ± 0.21	42.32 ± 1.5	36.06 ± 4.51	64.52 ± 4.98

Statistical Analysis: In *in-vivo* activity, the values are expressed regarding Mean ± S.E.M. of Prism 3.0 software was used to perform all statistical analysis, It was carried out by one –way analysis of variance (ANOVA), followed by Tukey Kramer multiple comparison post test. P values <0.05 were considered as significant.

RESULTS: Preliminary phytochemical screening revealed the presence of saponin, carbohydrate, glycosides, flavonoids, tannins and phenolic compounds.

Acute Toxicity Study: During acute toxicity studies MEME (2000 mg/kg.p.o.) neither produced any abnormal effect nor moribund stages. Moreover, no death was observed for 14 days among these animals.

Wound Healing Assessment: The studies on excision wound healing model revealed that all the three groups showed day to day decrease in the wound area. However, on 18th post-wounding day, control animals group-I showed 13.03 ± 1.2 of wound area whereas group-II Betadine treated an animal, showed 5.740 ± 0.10 wound area and the treated group-III exhibited 4.85 ± 1.02 wound area. When compared with the control, the activity of the extract was found to be highly significant (P<0.05).

***In-vivo* Antioxidant Assessment:** The study deals with the biochemical estimation of *Mimusops elengi* extract (500 mg/kg) in blood serum. The MEME has been found to reduce the serum ALP and Bilirubin in the treated groups compared to untreated ones. Treatment with CCl_4 increases the levels of total lipid, total triglycerides and total cholesterol in the liver which was reduced on the administration of the methanolic extract of *Mimusops elengi.*

DISCUSSION: The wound contraction of standard and extract ointment treated groups were found to be significant in comparison to simple ointment base treated group. Standard ointment treated wound was completely healed while extract ointment treated wound was almost at complete healing stage. It was observed that epithelialization period of the treated and standard group was less in comparison to simple ointment base treated group. The time required for complete epithelialization of the excision wound is an important parameter to

assess the wound healing process. Wound healing process consists of different phases such as contraction, epithelialization, granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent of each other. Hence in this study, excision wound model was used to assess the effect of herbal ointment on various phases. The result showed that methanolic extract ointment possesses a definite healing action.

The study showed that the *Mimusops elengi* had been found to reduce serum AST, ALT, ALP and Bilirubin. Treatment with CCl_4 increases the levels of total lipid, total triglycerides and total cholesterol in the liver. It has been established that CCl_4 accumulates in hepatic parenchymal cells and gets metabolically activated by CYPP-450 depended on monooxygenases form trichloro-methyl free radical (CCl_3).

Presence of significantly high concentration of total lipid and cholesterol in the serum of CCl_4 treated animals and maintains of these towards near normal values in methanolic extract administered rats demonstrates the hepatoprotective effect of *Mimusops elengi* leaves.

CONCLUSION: It can be concluded from the study that the methanolic leaf extract of *Mimusops elengi* Linn. (Sapotaceae) justifies the pronounced wound healing and antioxidant effect.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:
Gupta S, Irchhaiya R, Singh N, Gupta A, Alok S and Sinoriya A: Wound healing and *in-vivo* antioxidant activity of *Mimusops elengi* Linn. leaf extract. Int J Pharmacognosy 2014; 1(3): 200-06. doi: 10.13040/IJPSR.0975-8232.1(3).200-06.