ISSN 2449-8947

Volume 4
Number 1
January-June 2016

MicroMedicine

formerly
Archives of Biomedical Sciences

MNiSW points 2015: **8**
Index Copernicus 2014: **91.01**

http://www.journals.tmkarpinski.com/index.php/mmed
e-mail: mmed@interia.eu

MicroMedicine

ISSN 2449-8947

List of Peer-Reviewers
http://www.journals.tmkarpinski.com/index.php/mmed/pages/view/reviewers

Author Guidelines
http://www.journals.tmkarpinski.com/index.php/mmed/about/submissions

More information
www.journals.tmkarpinski.com/index.php/mmed

Publisher and Editor's office: Tomasz M. Karpiński, Szkółkarska 88B, 62-002 Suchy Las, Poland, e-mail: mmed@interia.eu

Contents

ISSN 2449-8947 MicroMedicine Research Article

Immunosuppressive activity of crude terpenoids from extracts of *Emblica officinalis*, *Ficus racemosa* and *Strychnos nux-vomica*

Amit Gupta[1]*, Sushama R. Chaphalkar[2]

[1]Department of Immunology, Vidya Pratishthan's School of Biotechnology, Vidyanagari, Baramati, District Pune, Maharashtra, India

[2]Director, Vidya Pratishthan's School of Biotechnology, Vidyanagari, Baramati, District Pune, Maharashtra, India

* Corresponding author: Dr. Amit Gupta, Assistant Professor (Immunology); e-mail: amitrrl@yahoo.com; amitgupta@vsbt.res.in

Received: 30 December 2015; **Revised submission:** 28 January 2016; **Accepted:** 03 February 2016

ABSTRACT

According to the literature which revealed that immunopharmacological based activity of crude terpenoids extracted from the leaves of *Emblica officinalis*, *Ficus racemosa and Strychnos nux-vomica* on human whole blood has not been carried out. In order to explore its immunosuppressive activity of these crude terpenoids (6.25-25 mg/ml; 50 µl) using human whole blood stimulated with hepatitis B vaccine containing surface antigen (HBsAg, 20 µg/ml; 10 µl) pertaining to human blood counts (lymphocytes, monocytes and granulocytes count), CD14 monocyte surface marker and HBsAg stimulated proliferation assay. The results showed that these crude terpenoids extracted from various medicinal plants containing HBsAg inhibited the percentage count of monocytes as well as granulocytes at higher doses. In addition, these crude terpenoids also reduces its proliferation rate at higher doses as compared to control and standard (HBsAg). The overall conclusion of this study showed that these crude terpenoids possess immunosuppressive activity and might be used for various therapeutic purposes.

Keywords: *Emblica officinalis*; *Ficus racemosa*; *Strychnos nux-vomica*; Hepatitis B vaccine.

1. INTRODUCTION

One of the largest groups of medicinal plant products i.e. terpenoids occupied an important position with more than twenty three thousands structures are known and they are also referred as terpenes [1]. Out of these compounds, most of them are volatile substances which provide fragrance to their plant including flowers and are generally used in industries especially for perfumery and cosmetic products including food additives [2, 3]. All these terpenoids are widely distributed as well as reported in leaves and fruits of medicinal plants including conifers, citrus etc with general formula $(C_5H_8)_n$ and classified as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$) and sesterterpenes ($C_{25}H_{40}$). On the basis of this classification, these terpenoids displayed a wide range of immunobiological activities against cancer, malaria, inflamma-

tion etc. [4, 5]. According to literature, there are number of terpenoids which were used for various immunobiological activities such as limonene (monocyclic monoterpene; major constituent of peel oil, oranges, citrus, lemon etc); sesquiterpene lactone (present in species i.e. *Pyrethrum parthenium, Leucanthemum pathenium* etc. and also present in anti-malarial drug compound *Artemisinin* isolated from *Artemisia annua*); diterpenes e.g. acyclic diterpenes, *Phytol*, biomarker found in aquatic environment and side chain of chlorophyll-a [6, 7].

As per the Indian traditional system of medicine (Ayurveda), *Emblica officinalis* (Amla; family *Euphorbiaceae*), showed various immunopharmacological activities in animal model (preclinical) studies i.e. immunomodulatory, anti-inflammatory, anti-cancer etc. and also showed its effectiveness against various disorders (heart, eye, respiratory, diabetes etc.) [8-10]. The most popular or effective plant part i.e. fruit which is generally used to treat many ailments e.g. common cold, fever etc. As per the literature, this medicinal plant is reported as a dietary source of vitamin C, minerals and amino acids. In addition, this medicinal plant protects the cells against free radical damage and provides anti-oxidant protection but it promotes as disease preventing tonic [11]. Number of studies has been shown that this medicinal plant used as immunity booster i.e. there is significantly enhancement of white blood cells count and also enhanced its immunity reported in rodent model studies [11-13].

Ficus racemosa (Umber; family *Moraceae*) commonly found in forests and water streams and showed numerous medicinal uses i.e. bark (skin diseases, anti-diabetic and anti-septic agents); leaves (mixed with honey are given in bilious infections); roots (dysentery, diabetes, hydrophobia etc.); fruits (blood disorder, fatigue, leprosy, scabies etc.) and latex (diabetes, toothache, vaginal disorders etc.) [14-16] and showed various immunopharmacological activities such as anti-cancer, gastroprotective, wound healing, cardioprotective, hepatoprotective, anti-pyretic, antidiuretic, anti-inflammatory, anti-bacterial, memory enhancing, antioxidant, hypoglycemic etc. As per the literature, tetracyclic triterpene (glauanol acetate) were isolated as well as purified from the leaves including stem bark and fruit and also reported protease (aspartic) from the latex of the plant. In addition, the leaves of this medicinal plant showed the presence of sterols and triterpenoids using petroleum ether extract and also determined qualitatively i.e. alkaloids, tannins and flavonoids using ethanolic extract [17, 18].

In addition, *Strychnos nux-vomica* (kajara, kuchala; family *Loganiaceae*) medicinal plant found in India and showed its immunobiological effect against certain disorders e.g. diabetes, asthma etc. As per the literature, two alkaloids i.e. brucine and strychnine isolated from medicinal plant and showed anti-cancer, anti-inflammatory, analgesic activities etc. [19-21]. As per the literature, this medicinal plant is recommended for heart and eye diseases, nervous disorders, respiratory diseases in elderly person etc and also reported as poison when consumed in large doses producing titanic convulsions and eventually leads to death [20, 21].

In the present study, little information is provided about the role of secondary metabolites especially terpenoids isolated from the leaves of these medicinal plants and supports the use of these terpenoids as an immunopharmacological agent against specific protein antigen (hepatitis B vaccine, HBsAg) using human whole blood.

2. MATERIALS AND METHODS

2.1. Collection of fresh plant material

Fresh plant leaves of medicinal plants i.e. *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* were collected from the garden of Vidya Pratishthan's School of Biotechnology, Baramati, District Pune, Maharashtra, India.

2.2. Identification and extraction of terpenoids

Fresh plant leaves of *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* were washed thoroughly three to four times with running tap water and then with distilled water. Finally, this plant material was dried in a shady area and prepared to form fine powder. For preliminary studies, this plant dried material is used for analyzing the secondary metabolite i.e. terpenoid using methanol, chloroform and sulphuric acid. For quantitative based studies, dry leaves powder (1 g) was taken into a test tube and adds 10 ml of

methanol was poured in it. Afterwards, the plant material was shaken and collects the filtrate. Thereafter add 2 ml and 3 ml of chloroform and sulphuric acid were mixed in extract of selected plant samples of *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica*. Formation of reddish brown color indicates the presence of terpenoids in the selected plants [22].

For isolation of terpenoids from medicinal plants, weigh 5 g of plant leaves powder of *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* were taken separately and soaked in alcohol for 24 hours. Thereafter collect the filtrate from leaves powder and was extracted with petroleum ether using separating funnel. The ether extract was treated as total terpenoids [23].

2.3. Human blood samples and estimates its blood counts

Non-infected human EDTA blood samples were collected from Mangal Pathology laboratory, Baramati region, District Pune, Maharashtra, India. For these studies, 100 µl of EDTA human whole blood were taken in 3 ml falcon tube and then add serial dilutions of crude terpenoids (6.25-25 mg, 50 µl) including hepatitis B vaccine (HBsAg; 20 µg/ml, 10 µl). Incubate whole blood samples containing serial dilutions of crude terpenoids along with standard HBsAg for 2 h. After incubation, lysed human blood samples using red cell lysis buffer (ammonium chloride, potassium bicarbonate and ethylene diamine tetraacetic acid) and washed the samples two times with phosphate buffered saline (PBS, pH 7.2) and proceed for flow cytometric analysis for the estimation of total blood (lymphocytes, monocytes and granulocytes) counts in human whole blood [10, 24, 25].

2.4. Estimation of CD14 FITC monocyte surface marker

In this study, human lysed whole blood samples were cultured in 96 well flat bottom tissue culture plate for 24 h in carbon dioxide incubator (37 °C, 5% CO_2) along with variable doses of crude terpenoids (6.25-25 mg, 50 µl) extracted from *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* along with HBsAg (20 µg/ml; 10 µl). After incubation, collect the cells after centrifugation of samples and stained with CD14 FITC (3 µl) monocyte surface marker. Incubate the samples for 30 minutes in dark at room temperature. After incubation, wash (2500 rpm; 10 min) the samples two times with PBS (pH 7.2) and proceed for flow cytometric analysis [10, 24, 25].

2.5. Cytotoxicity assay

Cytotoxicity assay was performed in lysed human whole blood and was cultured for 48 h in presence of variable doses of crude terpenoids (6.25-25 mg/ml, 50 µl) along with standardized dose of HBsAg (10 µl) in 96 well flat bottom tissue culture plates. After 48 h incubation, add MTT solution (2.5 mg/ml; 10 µl) and incubate 96 well plates for 2-3 h in carbon dioxide incubator. Again, the plates were centrifuged (1800 rpm for 5 minutes) and supernatant was eliminated. Add DMSO (100 µl solution) to the formazon crystals and the absorbance was evaluated in an ELISA reader at 570 nm [24, 25].

2.6. Statistical analysis

All values are mentioned as Mean ± S.E. The difference between the control and treated groups of terpenoids (6.25-25 mg, 50 µl) extracted from *Emblica officinalis, Ficus racemosa* and *Strychnos nux-vomica* containing HBsAg. Data is represented by One way ANOVA (Boniferroni multiple comparison) test.

3. RESULTS

3.1. Effect of terpenoids on human blood counts

To determine its effect using variable doses of crude terpenoids (6.25-25 mg/ml, 50 µl) on human blood counts as shown in Fig. 1. The results showed that these crude terpenoids showed decline at higher doses against HBsAg in the number of monocytes as well as granulocytes count. Overall, the results showed that these terpenoids extracted from *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* at higher doses against HBsAg showed immunosuppressive activity.

3.2. CD14 monocyte surface marker

To determine its effect using variable doses of crude terpenoids (6.25-25 mg/ml, 50 µl) on CD14 monocyte surface marker as shown in Fig. 2. The results showed that these crude terpenoids at higher doses extracted from *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* showed decline in CD14 monocyte surface marker as compared to standard (enhancement of CD14) and control.

3.3. Antigen (HBsAg) specific immune response

In order to observe its effect using variable doses of terpenoids on antigen (HBsAg) specific immune response as shown in Fig.3. The results showed that these terpenoids at higher doses showed decline in HBsAg specific immune response as compared to standard and control. Overall, the results confirmed its immunosuppressive activity against HBsAg.

4. DISCUSSION

In recent years, focus on the use of medicinal plant products in order to prevent and treat number of animal including human diseases has been revived all over the world. Most of the evidences collected from the literature which are based on medicinal plant products and showed immense potential against number of pathogens. Previously, number of medicinal plant products used and proved as immunomodulating agent against non specific antigen using sheep red corpuscles (SRBC) and are being extensively explored for their potential in the treatment and prevention of chronic diseases [26, 27]. In the present study, our research group focused on secondary metabolite i.e. terpenoids which are clinically useful and safe products from *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica* in human whole blood that could modulate the immune response. There are numerous examples in animal model studies including human whole blood and peripheral blood mononuclear cells where modulation of the antigen (HBsAg) specific proliferation including blood counts and CD14 monocyte surface marker alters the outcome of the diseases. These trends indicate that there is an urgent need for those compounds that can modulate or suppressed the production of antigen (HBsAg) specific proliferation, CD14 monocyte population etc.

Although the exact mechanism of this crude mechanism of terpenoids extracted from the leaves of *Emblica officinalis, Ficus racemosa* and *Strychnos nux-vomica* is not known, it may be mediated by the interaction between the most active components of crude terpenoids from these medicinal plants and cell-surface markers including growth factors involving specific protein antigen (HBsAg) activation, and it is possible that identification and elucidation of the active constituents in this crude terpenoids may provide beneficial leads to the development of new and effective immunosuppressant drugs. Immunopharmacological activity of crude terpenoids can be measured by detecting the changes in the human blood counts including CD14 monocyte surface marker and HBsAg proliferation assay. To elucidate the immunobiological effect of crude terpenoids on lysed human whole blood, our group measured the changes in blood counts, CD14 marker and proliferation assay.

In this study, our group evaluated the effect of these crude terpenoids from three medicinal plants i.e. *Emblica officinalis, Ficus racemosa* and *Strychnos nux-vomica* against HBsAg on human blood counts, CD14 monocyte surface marker using flow cytometry and HBsAg specific proliferation to determined its cytotoxicity. The outcome of the results proved that these terpenoids extracted from the leaves of these medicinal plants against HBsAg showed significantly suppress the production of human blood counts (monocytes as well as granulocytes count). Whether it's suppressive effect on the monocytes as well as granulocytes count was a direct result of its action on T cell stimulator (HBsAg). In order to confirm its activity against HBsAg, again the results showed that at higher doses, these crude terpenoids showed decline in HBsAg specific proliferation and CD14 monocyte surface marker as compared to control and standard (HBsAg). As per the studies, the results showed that these crude terpenoids showed highly significant and dose-dependent inhibition and reported immunosuppressive activity.

According to the literature, CD14 marker (55 kDa, glycoprotein; leucine-rich repeats) present abundantly on the surface of monocytes and macrophages and also reported in granulocytes

(neutrophils), basophils, Kupffer cells, dendritic cells and lymphocytes [28, 29]. But these markers will be detected only through flow cytometry. In the present study, our studies correlates CD14 monocyte surface marker with human blood counts (lymphocytes, monocytes and granulocytes count). The flow cytometric results clearly indicates that these crude terpenoids at higher doses showed immunosuppressive effect against HBsAg i.e. decline in monocytes as well as granulocytes count and CD14 monocyte surface marker. In addition, cell viability and HBsAg specific based assays are generally used for screening the crude terpenoids and the results indicates its immunosuppressive effect after incubating with optimized dose of HBsAg at higher doses as compared to control. Overall, the data showed its immunosuppressive activity.

5. CONCLUSION

Studies on cell-mediated immune response with respect to HBsAg specific proliferation, estimation of blood counts including CD14 monocyte cell surface marker which clearly indicates the immunosuppressive effects of crude terpenoids extracted from the leaves of *Emblica officinalis, Ficus racemosa and Strychnos nux-vomica*.

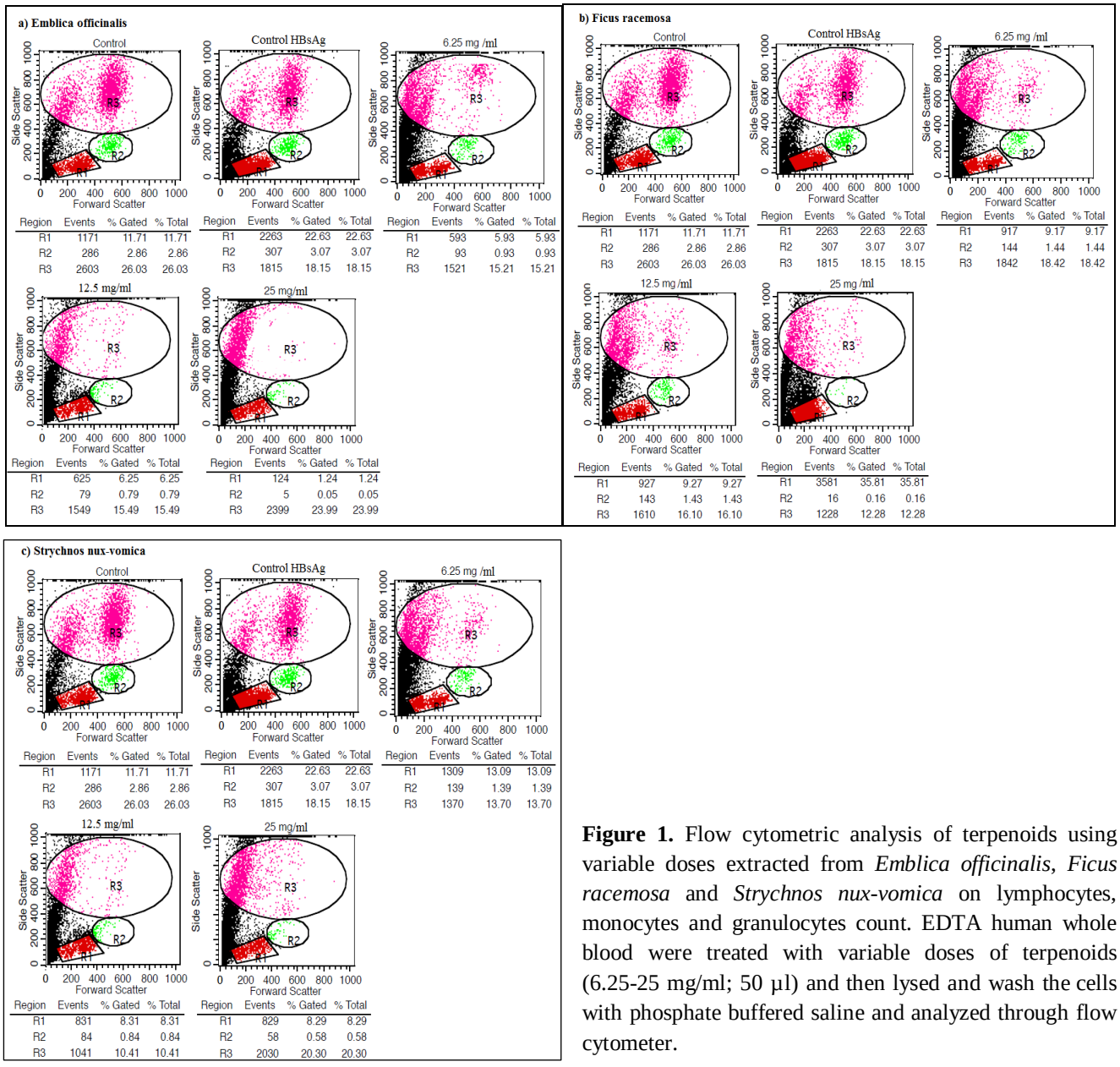

Figure 1. Flow cytometric analysis of terpenoids using variable doses extracted from *Emblica officinalis*, *Ficus racemosa* and *Strychnos nux-vomica* on lymphocytes, monocytes and granulocytes count. EDTA human whole blood were treated with variable doses of terpenoids (6.25-25 mg/ml; 50 µl) and then lysed and wash the cells with phosphate buffered saline and analyzed through flow cytometer.

In this study, crude terpenoids fraction at higher doses showed rapidly decline in the number of blood counts, especially monocytes and granulocytes count including CD14 monocyte marker and also represent inhibitor of antigen-specific immune responses. The findings demonstrate it to have a potent immunosuppressive potential, which is suggestive of its possible therapeutic usefulness. On the basis of this objective, it can be concluded that these terpenoids could be a potent immunosuppressive agent for next generation.

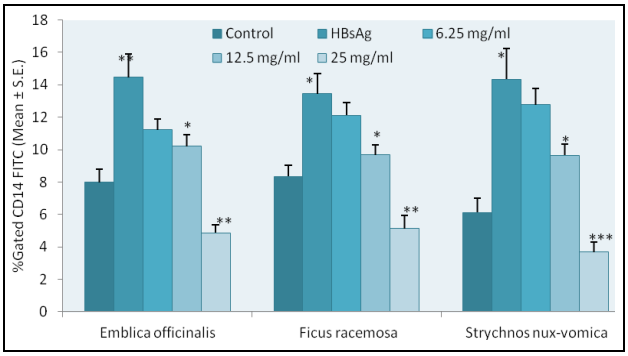

Figure 2. Effect of variable doses of terpenoid (6.25-25 mg/ml, 50 µl) on CD14 FITC monocyte surface marker in human whole blood using flow cytometry. Staining of human whole blood with CD14 FITC monocyte surface marker and then lysed and washed the samples with PBS. Finally, the samples were analyzed the samples through flow cytometer. Values are expressed as Mean ± S.E. The difference between the controls versus HBsAg versus variable doses of terpenoid is determined by one way ANOVA test (Boniferroni multiple comparison test).

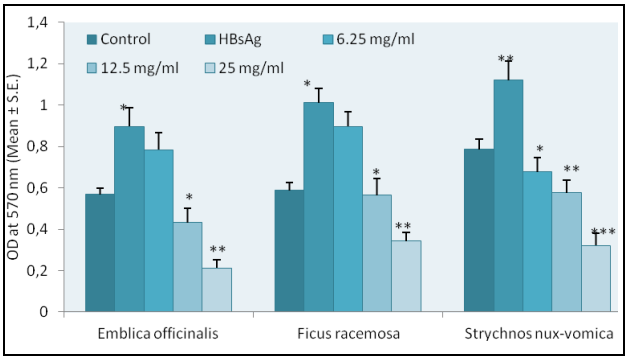

Figure 3. Effect of variable doses of terpenoid (6.25-25 mg/ml, 50 µl) on HBsAg specific proliferation in lysed human whole blood. Values are expressed as Mean ± S.E. The difference between the controls versus HBsAg versus variable doses of terpenoid is determined by one way ANOVA test (Boniferroni multiple comparison test).

AUTHORS' CONTRIBUTION

This work was carried out in collaboration between two authors. AM and SRC designed the study, wrote the protocol and interpreted the data; managed the literature searches and produced the initial draft. AM anchored the field study, gathered the initial data and performed preliminary data analysis. Both the authors read and approved the final manuscript.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

REFERENCES

1. Ourisson G, Nakatani Y. The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Curr Biol. 1994; 1: 11-23.
2. Zhang H, Qiu M, Chen Y, Yun S, Wang C, Harry HS, Fong HS. Phytochemistry and pharmacognosy - plant terpenes. Encycl Life Supp Systems (EOLSS). 1987; 4: 377-397.
3. Sacchettini JC, Poulter CD. Creating isoprenoid diversity. Science. 1997; 277(5333): 1788-1789.
4. Withers ST, Keasling JD. Biosynthesis and engineering of isoprenoid small molecules. Appl Microbiol Biotechnol. 2007; 73(5): 980-990.
5. Penuelas J, Munne-Bosch S. Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci. 2005; 10(4): 166-169.
6. Langenheim JH. Higher plant terpenoids: A phytocentric overview of their ecological roles. J Chem Ecol. 1994; 20: 1223-1280.
7. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochem. 1997; 44: 1185-1236.
8. Pardeshi S, Dhodapkar R, Kumar A. Molecularly imprinted microspheres and nanoparticles prepared using precipitation polymerisation method for selective extraction of gallic acid from *Emblica officinalis*. Food Chem. 2014; 146: 385-393.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi, India: CSIR; 2002.
10. Gupta A, Chaphalkar SR. Flow cytometric analysis of immunoadjuvant activity of *Emblica officinalis* on human whole blood. World J Pharmac Res. 2015; 4(2): 1063-1071.
11. Saeed S, Tariq P. Antibacterial activities of *Emblica officinalis* and *Coriandrum sativum* against Gram

negative urinary pathogens. Pak J Pharm Sci. 2007; 20(1): 32-35.

12. Sidhu S, Pandhi P, Malhotra S, Vaiphei K, Khanduja KL. Beneficial effects of *Emblica officinalis* in l-arginine-induced acute pancreatitis in rats. J Med Food. 2011; 14: 1-2.
13. Ganju L, Karan D, Chanda S, Srivastava KK, Sawhney RC, Selvamurthy W. Immunomodulatory effects of agents of plant origin. Biomed Pharmacother. 2003; 57(7): 296-300.
14. Gupta A, Chaphalkar SR. Flow cytometric immunopharmacological studies of aqueous extract of *Ficus racemosa* as an immunomodulator. J Int Res Med Pharmac Sci. 2016; 6(3): 108-114.
15. Paarakh PM. *Ficus racemosa* Linn - an overview. Nat Prod Radiance. 2009; 8: 84-90.
16. Chandrashekhar CH, Latha KP, Vagdevi HM, Vaidya VP. Anthelmintic activity of the crude extracts of *Ficus racemosa*. Int J Green Pharm. 2008; 2: 100-103.
17. Agarwal S, Misra K. Leucoanthocyanins from *Ficus racemosa* bark. Chemica Scripta. 1977; 12: 37-39.
18. Bhatt K, Agarwal YK. Chemical investigation of the trunk bark from *Ficus racemosa*. J Indian Chem Soc. 1973; 50: 611-613.
19. Duddukuri GR, Brahmam AN, Rao DN. Suppressive effect of *Strychnos nux-vomica* on induction of ovalbumin-specific IgE antibody response in mice. Indian J Biochem Biophys. 2008; 45: 341-344.
20. Gupta A, Chaphalkar SR. Cytotoxic and anti-inflammatory activity of aqueous extract of *Strychnos nux-vomica*. J Biol Nature. 2015; 4(4): 217-223.
21. Rao PS, Ramanadham M, Prasad MN. Anti-proliferative and cytotoxic effects of *Strychnos nux-vomica* root extract on human multiple myeloma cell line - RPMI 8226. Food Chem Toxicol. 2009; 47: 283-288.
22. Ramani R, Sharma KK, Lakhanpaul S, Kaushik S, Pushker AK. Preliminary phytochemical investigation on the bark of some of the important host plants of *Kerria lacca* - the Indian lac insect. Bot Res Int. 2011; 4: 48-51.
23. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R, Asnad. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem. 2013; 2(4): 1-4.
24. Gupta A, Chaphalkar SR. Immunopharmacological activity of *Zingiber officinale* on human peripheral blood mononuclear cells. Asian J Med Pharmac Res. 2015; 5(2): 13-17.
25. Gupta A, Jagtap RB, Chaphalkar SR. Flow cytometric evaluation of anti-viral activity of *Aegle marmelos* against Newcastle disease virus. Int J Res Pharm Life Sci. 2015; 3(2): 283-287.
26. Puri SC, Amna T, Khajuria A, Gupta A, Spiteller M, Qazi GN. Immunomodulatory activity of an extract of the novel fungal endophyte *Entrophospora infrequens* isolated from *Nothapodytes foetida* (Wight) sleumer. Acta Microbiol Immunol Hung. 2007; 54(3): 237-260.
27. Gupta A, Khajuria A, Singh J, Bedi KL, Satti NK, Dutt P, et al. Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from *Picrorhiza kurroa*. Int Immunopharmacol. 2006; 6(10): 1543-1549.
28. Antal-Szalmas P, Strijp JA, Weersink AJ, Verhoef J, Van Kessel KP. Quantitation of surface CD14 on human monocytes and neutrophils. J Leukocyte Biol. 1997; 61: 721- 728.
29. Griffin JD, Ritz J, Nadler LM, Schlossman SF. Expression of myeloid differentiation antigens on normal and myeloid cells. J Clin Invest. 1981; 68: 932-941.

ISSN 2449-8947 MicroMedicine Research Article

Some less known folk medicine in Bhadrak and Kendrapara districts of Odisha, India

Taranisen Panda[1]*, Nirlipta Mishra[2], Bikram Kumar Pradhan[1], Rajballav Mohanty[3]

[1] Department of Botany, Chandbali College, Chandbali, Bhadrak - 756133, Odisha, India
[2] Department of Zoology, Chandbali College, Chandbali, Bhadrak - 756133, Odisha, India
[3] Department of Botany, N.C. (Autonomous) College, Jajpur - 755001, Odisha, India
* Corresponding author: Taranisen Panda; e-mail: taranisenpanda@yahoo.co.in

Received: 26 December 2015; **Revised submission:** 02 February 2016; **Accepted:** 08 February 2016

ABSTRACT

An ethnobotanical investigation was carried out in Bhadrak and Kendrapara districts of Odisha, India to explore the therapeutic use of plants by local inhabitants. Data on medicinal plants were collected from June 2012 to July 2014 using structured questionnaires, complemented by free interviews and informal conversations. The research resulted identification of 64 medicinal plants belonging to 45 families. The most cited families were Cucurbitaceae, Fabaceae, Araceae and Asteraceae. Different plant parts like bark, leaf, flower, seed, stem, root and whole plant were reported as used in raw or cooked form against different diseases. Prominent diseases treated by plant remedies were asthma, cardiovascular ailments, gastrointestinal disorders, diabetes, jaundice, malaria, skin disorders and rheumatism. Data obtained showed that in the studied area the folk use of plants is still alive and plays an important role in the conservation of biodiversity and the regional indigenous medicinal knowledge for future generations. Further investigation of the reported plant species related to pharmacological and phytochemical studies may lead to the discovery of new bioactive compounds for treating life-threatening illnesses.

Keywords: Folklore; Phytotherapy; Medicinal plants; Traditional knowledge.

1. INTRODUCTION

Folk medicine has been used in almost all cultures throughout the world and still the mainstay of about 80% of the present world population for their primary healthcare [1, 2]. Medicinal plants are the resource of drug of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical entities for synthetic drugs [3, 4]. According to an estimate of the WHO, about 25% of the drugs prescribed worldwide come from plants and a significant number of basic and essential life saving drugs are exclusively of plant origin [5]. The reason is that, some of the important drugs for some serious diseases which did not have suitable remedies in modern system have been developed from plants. These include the anti-hypersensitive reserpine from *Rauvolfia serpentine*; the anticancer vinblastine and vincristine from *Catharanthus roseus*; antimalaria quinine from *Cinchona ladgeriana*; atropine from *Atropa belladonna*; the pain killer morphine and the anti-cough codeine from *Papaver somniferum* etc. Furthermore, compounds

such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies [6].

Several studies have been made on plants under a multitude of ethnobotanical grounds. According to a recent study conducted by the Ministry of Environment and Forest [7], Govt. of India, New Delhi, under the "All India Coordinated Research Project on Ethnobiology", the ethnic communities in India use over 10,000 wild plant species for meeting their primary health care, food and material requirements. About 8000 wild plant species are used in traditional, folk and herbal medicine for a variety of medicinal purposes, which covers about 1, 75, 000 specific applications. These plants are representing about 75% of the medicinal needs of the Third World Countries [8].

In India, a large percentage of the population is found in rural areas where health is a major constraint. In these areas hundred of primary health centers which are intended to serve rural people, lack staff, diagnostic facilities, and adequate supplies of drugs. Inaccessibility to modern allopathic modalities, the decreasing efficacy of synthetic drugs and the increasing adverse effects of their usage [9, 10] have helped for herbal remedies which are safe and effective (WHO 2002), have minor or no side effect [11]. Research studies have also shown that, diets rich in fruits; vegetables and derived food products have health benefits against cardiovascular diseases and certain types of cancer [12]. In this context ethnomedicinal studies are still in the initial stage in most parts of the Bhadrak and Kendrapara district of Odisha, and studies made so far are not comprehensive [13, 14]. Hence, the present investigation was carried out among the local inhabitants in rural areas of the Bhadrak and Kendrapara district to survey, explore and document the therapeutic use of potential medicinal plants.

2. MATERIALS AND METHODS

2.1. Study area

The study area comprises two adjoining districts of Odisha (i.e. Bhadrak 20°43'-21°13'N and 86°6'-87° E and Kendrapara 20º 21'-20º 47' N and 86º 14'-87º 03' E) situated in central coastal plain zone of Odisha state, eastern India, south Asia (Fig. 1) and covers an area of 5149 km^2, with a population of 29.46 lakhs (2011 Census). The investigation was carried out in all the nine blocks (*viz*., Aul, Derabis, Garadpur, Kendrapara, Mahakalpada, Marshaghai, Pottamundai, Rajkanika and Rajnagar) of Kendrapara district and seven blocks (Basudevpur, Bhadrak, Bhandaripokhari, Banta, Chandbali, Dhamnagar and Tihidi) of Bhadrak district. The districts accounts for 3.31% of the state's territory and shares about 7.02% of the state's population. Most of its people live in villages (90.94%) and agriculture is their main occupation. The climate of the districts is warm and humid. Three distinct seasons are felt during the year. The rainy season (mid June to mid October), winter (mid October to February) and summer (March to mid June). The air temperature ranges from 38°C in summer to 13°C in winter, and the annual average rainfall is approximately 1550 mm. The districts are located in the deltaic region with close proximity to the Bay of Bengal. Obviously, it has all the features of a costal climate, i.e. saline weather, influence of coastal wind and cyclone proneness.

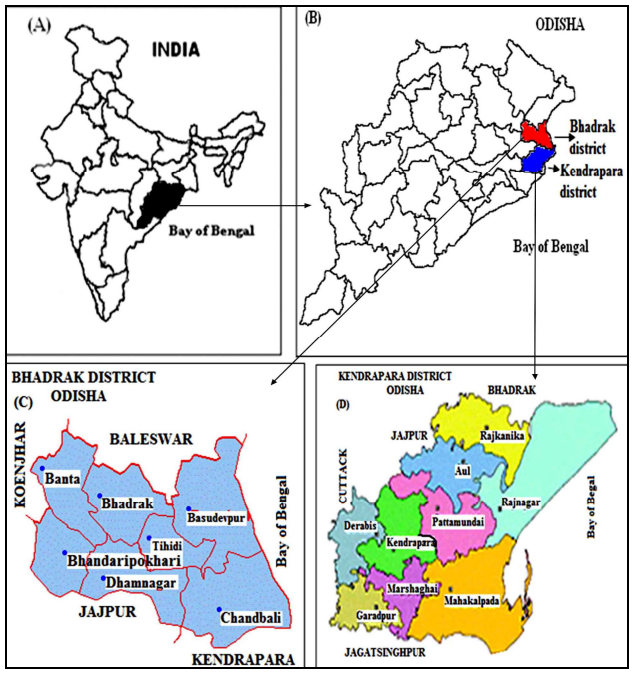

Figure 1. (A) Location of the Odisha state in the eastern region of India, (B) map of the Odisha state, (C) study area showing different blocks of the Bhadrak district, and (D) study area showing different blocks of the Kendrapara district.

2.2. Data collection

In order to document the utilization of indigenous medicinal plants, the field study was carried out monthly from June 2012 to July 2014 following established and standard procedures [15-17]. Before the field study: aims, methods, anticipated benefits of the study were adequately explained to the informants in local language, i.e. (Odia), due consent and cooperation was taken for the documentation of the traditional plants used by them. We discussed with the local healers, asked about their opinions and made decisions together on where to go and what to do in the coming days. The healers were the main informants, and their responses to our questions were recorded. One of us was responsible for writing down all the answers and information given by the healers. Structured questionnaires, complemented by free interviews and informal conversations were followed [18]. The experienced rural folk, traditional herbal medicine practitioners who were having knowledge of traditional healing were interviewed about the use of plants for different ailments. One hundred and thirty eight (105 men and 33 women) persons belonging to three different age groups were interviewed. Among these persons, 10% were at age of 21-40 years, 50% were at age of 41-60 years and the remaining 40% were 61 years or more. Surveys were conducted in different villages of the district**.** Information on vernacular names, life forms, growth, local status, growth ranges, sources, parts used, preparation, administration, and uses of the plant species were collected and recorded from the informants. Personal interviews and group discussions with local inhabitants revealed some valuable and specific information about the plants, which were further compared and authenticated by crosschecking [19]. Interviews with people were also conducted on a systematic basis to know more details about plant species, their management and distribution. The plant families, genera, and species of the collected specimens were identified by using standard floras and available literatures [20, 21]. The identified species were photographed and the voucher specimens were deposited in the herbarium of Botany Department, Chandbali College, Chandbali. The list of medicinal plants were depicted in a tabular form along with their botanical names followed by family, their local names in Odia if any and the parts used for medicinal purpose.

3. RESULTS AND DISCUSSION

The healers were observed to use a total of 64 plants distributed into 45 families for treatment of various ailments (Table 1; Figs. 2-5). The various ailments treated included fever, bone fracture, cardiovascular ailments, kidney disorders, loss of appetite, gastrointestinal disorders (such as diarrhoea and dysentery), diabetes, earache, lower abdominal pain during menstruation, jaundice, stomachache, burning sensations during urination, rheumatism and ulcer. The most cited families are Cucurbitaceae, Fabaceae, Araceae and Asteraceae. The great prevalence of plants belongs to Angiosperms (49 species to Dicotyledons and 14 species to Monocotyledons); the use of one Pteridophytes is also reported (Fig. 6). Herbs were the primary source of medicine (50%) followed by trees (21.9%), climbers (18.7%) and shrubs (9.4%) as shown in Figure 7. The common use of herbaceous medicinal plants were also reported in other parts of world [22-25], which may be due to easy availability and high effectiveness in the treatment of ailment in comparison to other growth forms [26]. Fifty two plants (81.3%) were categorized as wild while twelve plants (18.7%) were listed as cultivated plants (Fig. 8). Other research has also documented the dominance of wild plants in medicinal use [27-32]. Different plant parts like bark, leaf, flower, seed, stem, root and whole plant were reported as used in raw or cooked form. Leaves are predominantly used as a remedy for various ailments (21%), followed by fruit and roots (18.5%) each, seeds (9.9%), whole plant (9.9%), bark (7.4%) and the remainders were rhizome, flower, thorn, pulp and corm (Fig. 9). The common use of leaves have also been made in earlier studies [14, 25, 27, 29] which may be due to easy finding of this plant part and its availability during all seasons of the year and also contain high concentrations of active agents [33]. This practice helps to increase the chances of species survival and enhances the sustainable management of plants than the use of roots and/or the whole plant.

Figure 2. (a) *Acacia nilotica* (L.) Dilile, (b) *Amaranthus spinosus* L., (c) *Ananas comosus* (L.) Merr., (d) *Artocarpus lacucha* Roxb., (e) *Averrhoa carambola* L., (f) *Basella alba* L.

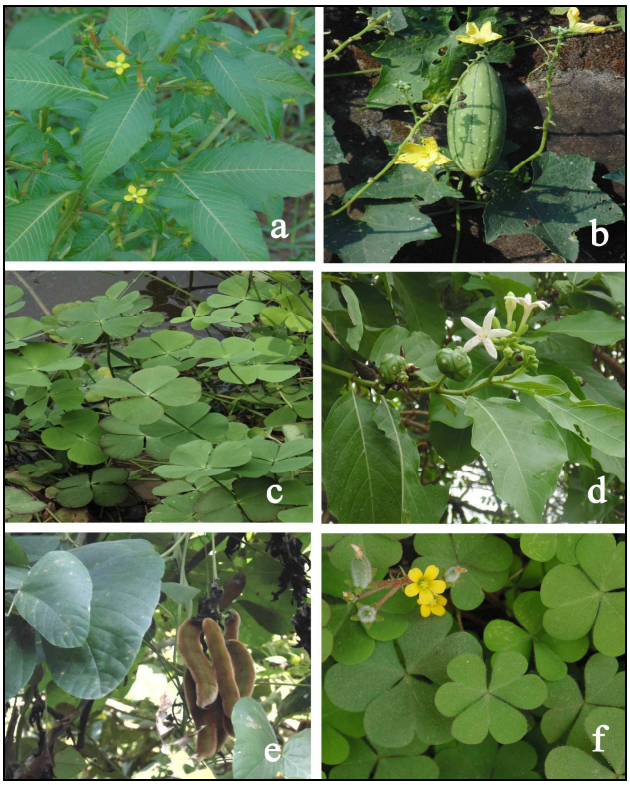

Figure 4. (a) *Ludwigia hyssopifolia* L., (b) *Luffa acutangula* L. var. amara Roxb., (c) *Marsilea minuta* L., (d) *Morinda citrifolia* L., (e) *Mucuna pruriens* L., (f) *Oxalis corniculata* L.

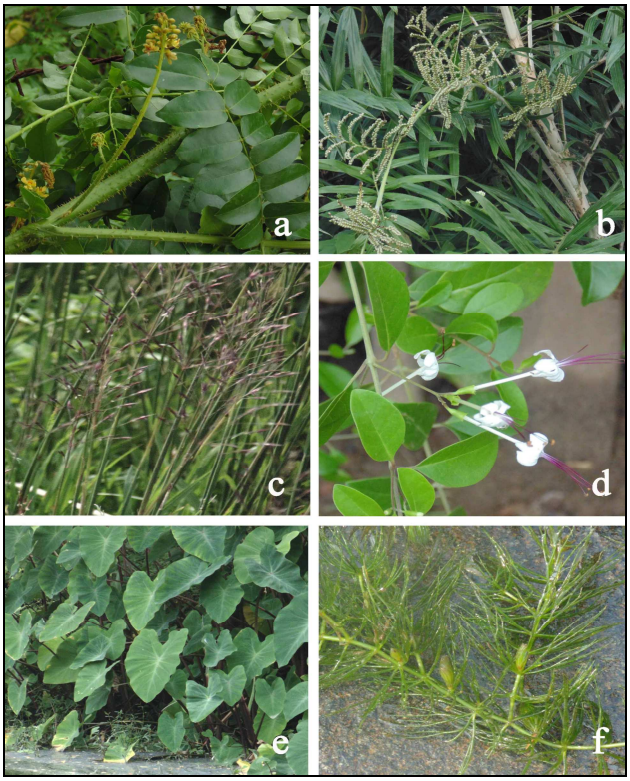

Figure 3. (a) *Caesalpinia bonduc* (L.) Roxb, (b) *Calamus rotang* L., (c) *Chrysopogon aciculatus* Retz. Trin., (d) *Clerodendrum inerme* L., (e) *Colocasia esculenta* (L.) Schott., (f) *Hydrilla verticillata* (L. f) Royle.

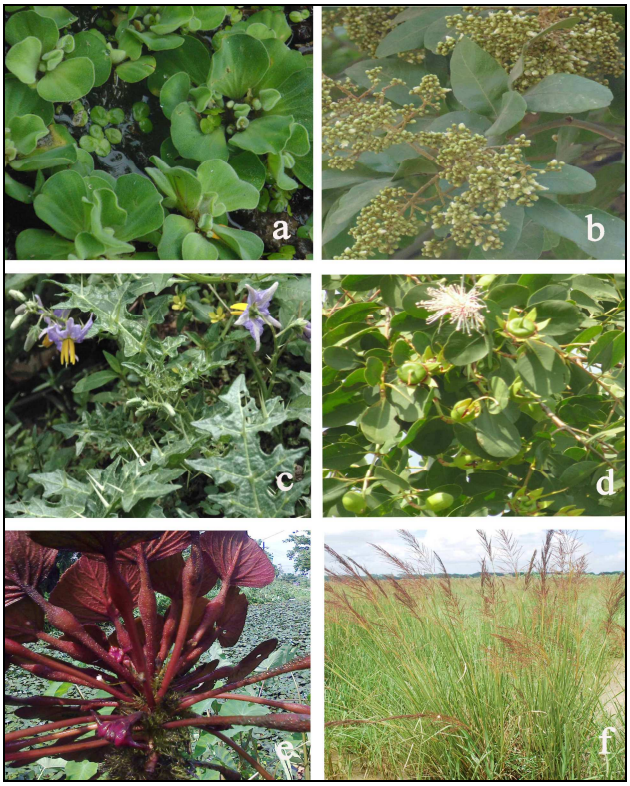

Figure 5. (a) *Pistia stratoites* L., (b) *Sapindus emarginatus* Bahl., (c) *Solanum virginianum* L., (d) *Sonneratia caseolaris* L., (e) *Trapa natans* L., (f) *Vetiveria zizanioides* (L.) Nash.

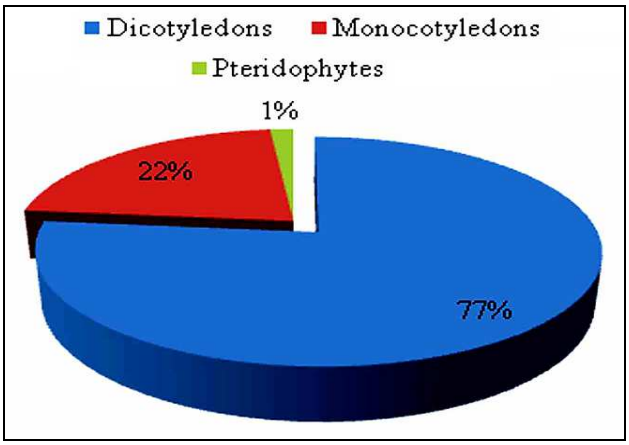

Figure 6. Proportions of plants used in the preparation of herbal medicines.

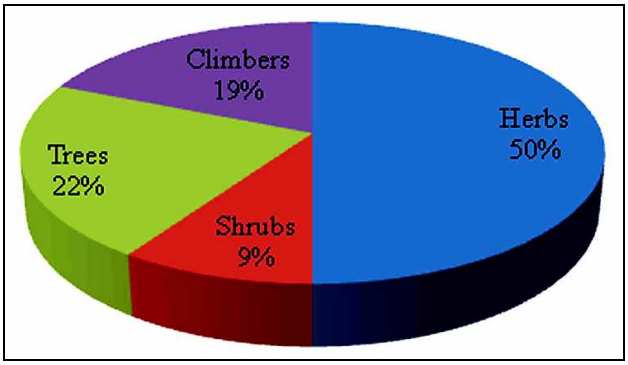

Figure 7. Percentage of growth forms used for medicinal purposes.

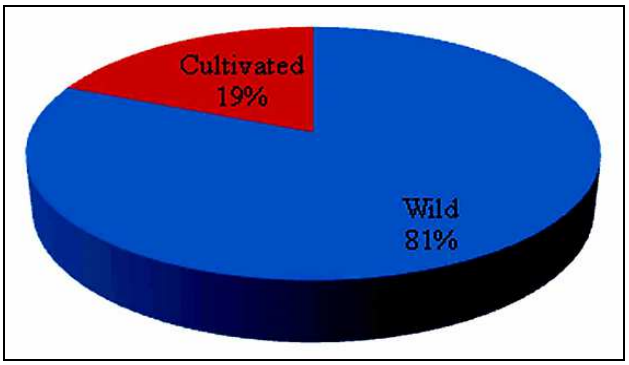

Figure 8. Distribution of wild and cultivated plants used in herbal medicine.

Some of the medicinal plant species reported in this paper were also documented in earlier works, but purposes and methods of uses are different, for example, tender leaves of *Acacia nilotica* are used as a gargle in spongy gums, sore throat and as wash in haemorrhagic ulcers and wound [34]; the juice of the bark mixed with milk is dropped into the eye in conjunctivitis [35]; decoction of the *Acorus calamus* is used for cholic [36]. Similarly, the root and fruit *Ananas comosus* are either eaten or applied topically as an anti-inflammatory and digestive [37]; *Artocarpus lacucha* bark chewed like betel nut is used to treat skin ailments [38]; the leaves of *Basella alba* are used to bring sound refreshing sleep when it is applied on head about half an hour before bathing [39]; *Cleome viscosa* roots are used for scurvy and rheumatism [40]. In the same way, the roots of *Clerodendrum inerme* is used to treat coughs and skin diseases [41]; the juice extracted from the leaf stalks of *Colocasia esculenta* with salt is used as an absorbent in cases of inflammed glands and buboes [42]; fruits of *Embelia ribes* is used for jaundice [43] and roots are used for Influenza [44]; whole plant of *Evolvulus alsinoides* is used to cure bronchitis, biliousness, epilepsy, leucoderma [45]. Likewise, the bark of *Feronia limonia* is prescribed for biliousness and liver diseases [46,47]; *Ipomea mauritiana* tuber root powder is reported to be of beneficial use to persons suffering from or prone to coronary disease problems and diabetes [48]; *Luffa acutangula* fruit is used for the treatment of diabetes [49]; *Marsilea minuta* is used in treatment of diarrhea, bronchitis, epilepsy, hepatitis, kidney infection [50]; green fruits of *Morinda citrifolia* is used for arthritis, gastric and oral ulcers, toothache, indigestion [51]; the fruits *Opuntia dillenii* is used for antinflammatory and analgesic effects [52]; roots of *Pandanus fascicularis* are widely used in treatment of osteoarthritis and skin diseases like leprosy [53,54]; *Pistia stratiotes* leaves are recommended for the treatment of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils, and wounds [55]; the roots of *Tragia involucrate* is used for antipyretic and wound healing [56] and the fruits of *Trapa natans* are recommended for use in bleeding disorders, threatened abortion, dysuria, polyuria and oedema [57].

The healers prescribed both single and multiple plant prescriptions for the treatment of various diseases. The use of single and multiple plant prescriptions have also previously been documented [25, 27, 58-60]. The paste and juice were the most usual forms of consumption of herbal medicine and the principal mode of administration was oral followed by external or topical application. Water is most often used as dilutant for the preparation of drugs followed by milk. Healers also

mix black salt, salt (sodium chloride) and sugar candy in herbal formulation and similar results were also previously reported [58, 61]. During the period of investigation the healers reported that the traditional knowledge acquired from their ancestors used to be transferred verbally to one of their successors.

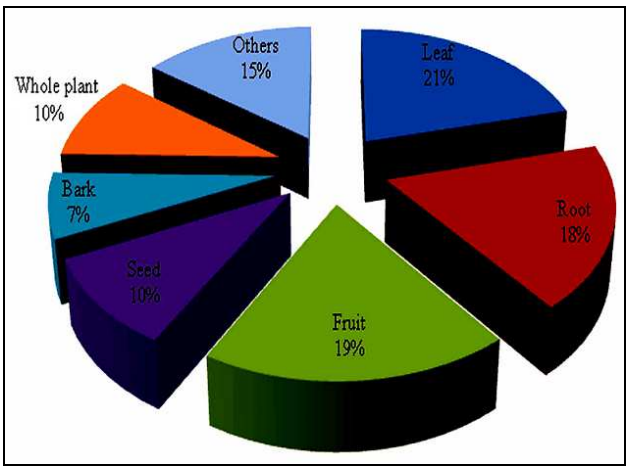

Figure 9. Percentage of relevant plant parts used in herbal medicine.

4. CONCLUSION

The present study indicates that the knowledge and usage of folk medicine still exists in rural areas of Bhadrak and Kendrapara districts, Odisha and play a significant role in the treatment of various human ailments and also testifies ancient traditions. Thus, from the modern social context and economic view of health services, it is imperative to link the folk medicine with the modern scientific knowledge related to pharmacological and phytochemical studies which may lead to the discovery of new drugs for treating life-threatening illnesses. Efforts should be made to cultivate potential medicinal plant species as field crops and their conservation should be done in appropriate ecological conditions.

ACKNOWLEDGEMENTS

The authors are grateful to the traditional healers concerned for sharing their traditional knowledge for documentation and photography.

AUTHORS' CONTRIBUTION

TP: Field data collection, compilation of data, collection of references, manuscript preparation, final editing and checking of manuscript. NM: Field data collection, compilation of data. BKM: Compilation of data, collection of references. RBM: Collection of references, manuscript preparation. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

REFERENCES

1. Kamboj VP. Herbal medicine. Curr Sci. 2000; 78: 35-39.
2. Owoabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella africana* (Bignoniaceae) stem bark. Afr J Biotechnol. 2007; 6: 882-885.
3. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol. 2008; 7(12): 1797-1806.
4. Singh JS. The biodiversity crisis. A multifaceted review. Curr Sci. 2002; 82(6): 638.
5. Rates SMK. Plants as source of drugs. Toxicon. 2001; 39: 603-613.
6. Williamson E, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant material. Wiley, Chichester, 1996.
7. Protect traditional knowledge, innovation and practices. Achieving 2010 Biodiversity Target: India's contributions: Maintain capacity of ecosystems to deliver goods and services and support livelihood. Goal 9. New Delhi: Ministry of Environment and Forest, Govt. of India, 2010.
8. Rajshekharan PE. Herbal medicine. In World of Science, Employment News. 21-27 Nov. 2002: 3.
9. Gijtenbeek JMM, Vanden BMJ, Vecht CJ. Cyclosporine neurotoxicity. J Neurol. 1999; 246: 339-346.
10. Johnson WC, William OW. Warfarin toxicity. J Vascular Surg. 2002; 35: 413-421.
11. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: Challenges, and opportunities to increase the

knowledge base for safety assessment. Toxicol Appl Pharmacol. 2010; 243: 198-216.

12. Maron DM, Ames BN. Revised methods for *Salmonella* mutagenicity test. Mut Res. 1983; 113: 173-215.
13. Girach RD, Aminuddin A, Brahman A, Mishra MK. Observations on ethnomedicinal plants of Bhadrak district, Orissa, India. Ethnobotany. 1997; 9: 44-47.
14. Panda T. Preliminary Study of ethno-medicinal plants used to cure different diseases in coastal district of Orissa, India. Brit J Pharmacol Toxicol 2010; 1(2): 67-71.
15. Jain SK, Goel AK. A manual of ethnobotany. Jodhpur, Scientific Publishers, 1995.
16. Maundu P. Methodology for collecting and sharing indigenous knowledge: a case study. Ind Know Dev Monitor. 1995; 3: 3-5.
17. Martin GJ. Ethnobotany - a manual of methods. London, Earthscan Publishers, 1995.
18. Huntington HP. Using traditional ecological knowledge in science: Methods and applications. Ecol Appl. 2000; 10(5): 1270-1274.
19. Cunningham AB. Applied ethnobotany, people wild plant use and conservation. London and Sterling VA, Earthscan Publishing, 2001.
20. Haines HH. The Botany of Bihar and Orissa. London: Adland and Son, 1925.
21. Saxena HO, Brahmam M. The Flora of Orissa. Vol 1-4. Bhubaneswar, Regional Research Laboratory, 1996.
22. Giday M, Asfaw Z, Elmqvist T, Woldu Z. An ethnobotanical study of medicinal plants used by the Zay People in Ethiopia. J Ethnopharmacol. 2003; 85: 43-52.
23. Tabuti JRS, Lye KA, Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. J Ethnopharmacol. 2003; 88: 19-44.
24. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J Ethnobiol Ethnomed. 2006; 2: 43.
25. Upadhyay PB, Roy S, Kumar A. Traditional uses of medicinal plants among the rural communities of Churu district in the Thar Desert, India. J Ethnopharmacol. 2007; 113: 387-399.
26. Rokaya MB, Münzbergová Z, Timsina B. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. J Ethnopharmacol. 2010; 130: 485-504.
27. Ayyanar M, Ignacimuthu S. Traditional knowledge of Kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. J Ethnopharmacol. 2005; 102: 246-255.
28. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities Chhota, Western Himalaya. J Ethnobiol Ethnomed. 2006; 2: 14.
29. Panghal M, Arya V, Yadav S, Kumar S, Yadav JP. Indigenous knowledge of medicinal plants used by Saperas community of Khetawas, Jhajjar District, Haryana, India. J Ethnobiol Ethnomed. 2010; 6: 4-13.
30. Cakilcioglu U, Khatun S, Turkoglu I, Hayta S. Ethnopharmacological survey of medicinal plants in Maden (Elazig-Turkey). J Ethnopharmacol. 2011; 137: 469-486.
31. Kadir MF, Bin Sayeed MS, Mia MMK: Ethnopharmacological survey of medicinal plants used by indigenous and tribal people in Rangamati, Bangladesh. J Ethnopharmacol. 2012; 144: 627-637.
32. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J Ethnopharmacol. 2013; 145: 554-565.
33. Vitalini S, Tomè F, Fico G. Traditional uses of medicinal plants in Valvestino (Italy). J Ethnopharmacol. 2009; 121(1): 106-16.
34. Nadkarni KM. The Indian plants and drugs. New Delhi: Shrishti Book Distributors, 2005: 4-5.
35. Said HM. Hamdard Pharmacopeia of Eastern Medicine. 2nd edn. New Delhi: Sri Satguru Publications, 1997.
36. Balakumbahan R, Rajamani K, Kumanan K. *Acorus calamus*: an overview. J Med Plants Res. 2010; 4(25): 2740-2745.
37. Bartholomew DP, Paull RE, Rohrbach KG. The pineapple: botany, production and uses. Wallingford UK: CABI Publishing, 2003.
38. Jagtap UB, Bapat VA. *Artocarpus*: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 2010; 129: 142-166.
39. Anandarajagopal K, Sudhahar D, Ajaykumar TV, Muthukumaran G. Evaluation of CNS depressant activity of aerial parts of *Basella alba* Linn. IJPI'S J Pharmacol Toxicol. 2011; 1: 5.
40. Rukmini C. Chemical, nutritional and toxicological evaluation of the seed oil of *Cleome viscosa*. Indian J Med Res. 1978; 67: 604-607.
41. Kanchanapoom T, Chumsri P, Kasai R, Otsuka H, Yamasaki K. A new iridoid diglycoside from

Clerodendrum chinense. J Asian Nat Prod Res. 2005; 7: 269-272

42. Kirtikar KR, Basu BD. Indian medicinal plants. Dehradun: International Book Distributors, 2005.
43. Kirthikar KR, Basu BD. Indian medicinal plants. Dehradun, International Book Distributors, Vol. II. 1987.
44. Menon K. *Embelia ribes* - a medicine for influenza. Indian Forester. 1999; 45: 210.
45. Sivarajan VV, Balachandran I. Ayurvedic drugs and their plant sources. New Delhi: Oxford Publishing, 1994.
46. Rajasab AH, Mahamad I, Documentation of folk knowledge on edible wild plants of North Karnataka. Indian J Trad Know. 2004; 3(4): 419-429.
47. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian medicinal plants. New Delhi: National Institute of Science Communication and Information Resources, 2002.
48. Moushumi SJ, Ahmed R, Ahmed H, Ali M, Haq WM, Jahan R, Rahmatullah M. Hypoglycemic, hypocholesterolemic and hypotriglyceridemic activity of tuber roots of *Ipomoea mauritiana* Jacq. (Convolvulaceae) when administered to rats. Adv Nat Appl Sci. 2010; 4: 174-176.
49. Pimple BP, Kadam PV, Patil MJ. 2011. Antidiabetic and antihyperlipidemic activity of *Luffa acutangula* fruit extracts in streptozotocin induced niddm rats. Asian J Pharma Clin Res. 2011; 4(Suppl. 2): 156-163.
50. Tiwari OP, Bhattamisra SK, Singh PN, Singh SK, Kumar V. Adaptogenic anti-stress activity of standardized extract of *Marsilea minuta* L. Pharmacologyonline. 2009; 1: 290-299.
51. McClatchey W. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). Int Can Thera. 2002; 1(2): 110-120.
52. Loro JF, Del Rio I, Perez-Santana L. Preliminary studies of analgesis and anti-inflammatory properties of *Opuntia dillenii* aqueous extract. J Ethnopharmacol. 1999; 67: 213-218.
53. Kapoor LD. Hand book of Ayurvedic medicinal plants. Lucknow, National Botanical Research Institute, 2001.
54. Sharma PC, Yelne MB, Dennis TJ. Database on medicinal plants used in Ayurveda. New Delhi: Dept. of ISM and Min. of Health and Family Welfare, 2001: 378-381.
55. Premkumar VG, Shyamsundar D. Antidermatophytic activity of *Pistia stratiotes*. Indian J Pharmacol. 2005; 37 (2): 126-128.
56. Nadkarni KM. Indian Meteria Medica. 3rd edn. Mumbai: Popular Prakashan, 1976.
57. Khare CP. Indian medicinal plants: illustrated dictionary. Berlin, Heidelberg, Springer, 2007.
58. Lal SD, Yadav BK. Folk medicines of Kurukshetra Districts (Haryana), India. Econ Bot. 1983; 37(3): 299-305.
59. Panda T, Panigrahi SK, Padhy RN. A sustainable use of phytodiversity by the Kandha tribe of Orissa. Indian J Trad Know. 2005; 4(2): 173-178.
60. Wodah D, Asase A. Ethnopharmacological use of plants by Sisala traditional healers in northwest Ghana. Pharmaceut Biol. 2012; 50(7): 807-815.
61. Asase A. Oppong-Mensah G. Traditional anti-malarial phytotherapy remedies in herbal markets in southern Ghana. J Ethnopharmacol. 2009; 126: 492-499.

Table 1. Ethnobotanical inventory of medicinal plants in Bhadrak and Kendrapara districts of Odisha, India.

Botanical name, family & local name	Growth forms	Parts used	Disease/condition	Mode of application
Acacia nilotica (L.) Delile.(Mimosaceae), 'Kanta Babula'	Tree	Leaf	Diabetes	Leaves are squeezed with water and the juice (5 ml) is taken for 30 days against diabetes.
Acorus calamus L. (Araceae), 'Bacha'	Herb	Leaf, rhizome	Insecticide asthma, bronchitis and cough	Dried leaves are used as insecticide. Fresh rhizome (2 g) is administered orally for 30 days to cure asthma, bronchitis and cough.
Alangium salvifolium (Linn. f.) Wang. (Alangiaceae), 'Ankula'	Tree	Leaf	Bone fracture, diabetes	Boiled leaves are made into paste and applied on the bone fractured areas till cure. Leaf leachate (10-15 ml) is taken for a week to reduce sugar in urine.
Amaranthus spinosus L. (Amaranthaceae), 'Kanta saga'	Herb	Leaf	Boils, burns	10 leaves are warmed gently and applied locally thrice daily for 5 days to cure boils and burns.
Amaranthus viridis L. (Amaranthaceae),	Herb	Whole plant	Ulcer	Whole plant is used as emollient for ulcer problems till cure.
Anacardium occidentale L. (Anacardiaceae), 'Saitamba'	Tree	Fruit	Diarrhea	Fruit juice (5-10 ml) is taken for three days to cure diarrhea.
Ananas comosus (L.) Merr. (Bromelliaceae), 'Sapuri'	Herb	Root, fruit	Anti-inflammatory digestive, diarrhea	The root and fruit are eaten raw as an anti-inflammatory and digestive. A root decoction (5-10 ml) is taken for 5-7 days against diarrhea.
Artocarpus lacucha Roxb. Buch. –Ham. (Moraceae), 'Jeutha'	Tree	Bark, fruit, seed	Skin, purgative, liver tonic	Bark powder when applied externally, draws out purulent matter; heals boils, cracked skin and pimples till cure. The seed are good purgative for children. The ripen fruit is used as tonic to liver.
Avicenia officinalis L. (Verbenaceae), 'Bani'	Tree	Fruit	Diabetes	Fruits are squeezed with water and the juice (5 ml) is taken for a month.
Averrhoa carambola L. (Oxalidaceae), 'Karmanga'	Tree	Flower, root, seed	Antihelmintic, malaria, asthma	The boiled flower juice (one spoonful) is taken for 3-5 days against helminthes, fever and malaria. Root paste with sugar is used as an antidote for poison. The powdered seed (2-3 g) is taken for 7 days to cure asthma.
Basella alba L. (Basillaceae), 'Poi'	Climber	Leaf, root	Gynaecology, constipation	Juice of the leaves (3-5 ml) with honey is given for 3 days in case of constipation particularly in children. The root paste along with rice washed water is taken in the morning in empty stomach for one month to cure irregular periods. Leaf is rubbed over the affected part to cure irritation and swellings due to caterpillar.
Bauhinia vahlii Wight. & Arn. (Caesalpiniaceae), 'Siali'	Climber	Bark	Dysentery	Barks of the stem and lime made into paste and are taken orally, twice daily for 3 days to cure dysentery.
Benincasa hipsida (Thumb) Cong. (Cucurbitaceae), 'Panikakharu'	Climber	Fruit	Diabetes	Fruit juice of about 20 ml along with 1 g seed powder of *Syzygium cumini* (L.) Skeels (Jamu) is taken twice daily for 3 months to control blood sugar.

Botanical name, family & local name	Growth forms	Parts used	Disease/condition	Mode of application
Bombax ceiba L. (Bombacaceae), 'Simili'	Tree	Thorn, root, flower	Gynecological disorder, urinary troubles, dysentery	Thorn paste with cow milk is applied on pimples. Root paste with unboiled cow milk is taken to regulate the menstruation and with black pepper it is used to control white discharge. Root decoction (about 50 g root boiled in a cup of water) is taken orally twice daily for a week to cure urinary troubles. Decoction of flowers (about 5 teaspoonfuls) is given twice daily for 3-4 days to cure dysentery.
Caesalpinia bonduc (L.) Roxb.(Caesalpiniaceae),' Gila'	Shrub	Leaf	Jaundice	Leaf paste (3-5 g) is taken for 7 days to cure jaundice.
Calamus rotang L. (Arecaceae), 'Beta'	Shrub	Bark	Dysurea	Juice of the fresh bark (50 g) is taken twice daily for a month against dysurea.
Cayratia trifolia (L.) Domin. (Vitaceae), 'Ambiliti'	Climber	Tuber, root	Snake bite, bone fracture	Paste of tuber applied on the affected part in case of snake bite. Powder of tuberous root (2-3 g) is taken orally with milk for early recovery from fractured bone.
Ceratophyllum demersum L. (Ceratophyllaceae), 'Panisiuli'	Herb	Flower	Glycosuria	The juice of the flower along with a teaspoon of lemon juice and pinch of common salt is taken for 2 months against glycosuria.
Chenopodium album L. (Chenopodiaceae), 'Bathuasaga'	Herb	Whole plant, leaf	Piles, cardiovascular	Leaf juice (3-5 ml) is administered for 21 days to cure piles. One spoonful of whole plant decoction is used for killing intestinal worms. Leaf is eaten for 30 days by the heart patient to get relief from heart diseases.
Chrysopogon aciculatus (Retz.) Trin. (Poaceae), 'Guguchia'	Herb	Rhizome	Stomach problem	Fresh rhizome along with 3-5 black pepper is crushed into a paste and taken early in the morning for one month in empty stomach for stomachache and gastric disorder.
Cicer arietinum L. (Fabaceae), 'Sola'	Herb	Leaf	Stomachache	One spoonful of leaf Juice (3-5 ml) is taken against stomachache and laxative for 3-5 days.
Cleome viscosa L. (Capparaceae), 'Banosorisho'	Herb	Root	Earache	Root is pounded with garlic, mixed with castor oil, boiled and about 2-3 drops of squeezed juice is applied in the ear to treat earache
Clerodendrum inerme (L.) Gaertn. (Verbenaceae)	Herb	Leaf	Rheumatism	The powdered leaves mixed with camphor, garlic or pepper is taken for a week to cure edema. Leaf juice is taken orally for 5-7 days to cure muscular pain as well as rheumatic pain. The root paste is used for venereal diseases.
Colocasia esculenta (L.) Schott. (Araceae)	Herb	Corm	Vigour & vitality	50 g of boiled corm is eaten once daily for one month.
Curcuma aromatica Salisb. (Zingiberaceae), 'Palua'	Herb	Rhizome	Diarrhea	Juice (3-5 ml) of the crushed rhizome is taken with honey for 3-5 days to cure diarrhea in children.
Diospyros malabarica (Desr.) Kostel. (Ebenaceae) , 'Dhusara kendu'	Tree	Bark	Cardiac problem	Decoction of the bark along with the *Syzygium cumini* (L.) Skeels (Jamu) is taken against high blood pressure.
Dregea volubilis (L.f.) Benth. ex Hook.f. (Strychnaceae)	Herb	Root	Pain in external injury	Root paste is applied topically to cure pain due to external injury.

Botanical name, family & local name	Growth forms	Parts used	Disease/condition	Mode of application
Embelia ribes Burn.f. (Myrsinaceae)	Climber	Fruit	Kidney problem	Fruit powder (1-2 g) mixed with 2-3 black pepper seeds in hot water is taken for 7 days to check frequent urination.
Euryale ferox Salisb. (Nymphaeaceae), 'Kantapadma'	Herb	Seed	Leucorrhoea	Young seeds are eaten to strengthen spleen, also effective for leucorrhoea, impotency.
Evolvulus alsinoides (L.) (Convolvulaceae) 'Bichhamalia'	Herb	Whole plant	Brain disorder	The whole plant extracts (5-10 ml) are taken for 21 days to treat brain disorders like insanity, epilepsy, and nervous debility.
Flacourtia indica (Burm.f.) Merr. (Flacourtiaceae), 'Bainchakoli'	Shrub	Stem-bark fruits, root	Eczema, urinary, fever	Infusion of crushed fruits is taken (150 ml) in the morning for three days in painful urination. 5 g stem bark paste is applied by rubbing the skin once a day for eczema. Bark paste (2 g) are taken for 3-5 days in case of intermittent fever.
Helianthus annus L. (Asteraceae), 'Surajyamukhi'	Herb	Seed	Bone fracture	Oil extracted from seed is applied for quick healing of bone fracture till cure.
Holarrhena pubescens (Buch-Ham) Wall.ex G.Don. (Apocynaceae), 'Kerua'	Tree	Root, bark	Snake bite, wounds, boils, blood dysentery	The root paste is applied on snakebite to cure snake bite. Water extract of bark is given for 3-5 days to cure blood dysentery. Root paste is applied on cut, wound, abscess and boils.
Hydrilla verticillata (L.f.) Royle. (Hydrocharitaceae), 'Chingudia dala'	Herb	Whole plant	Joint pain	Whole plant is slightly warmed and tied on the affected area to cure joint pain.
Lablab purpureus (L.)Sweet. (Fabaceae), 'Shimba'	Climber	Fruit	Diabetes	Tendered pods (10-20 g) are cooked and taken daily in diet for one month to control blood sugar.
Lagenaria siceraria (Molina) Standley (Cucurbitaceae), 'Laoo'	Climber	Pulp	Diabetes	Fruit pulp (5 g) is taken in empty stomach for 30-40 days to reduce blood sugar.
Limnophila heterophylla (Roxb.) Benth. (Scrophulariaceae)	Herb	Leaf	Ophthalmic disease	Leaf juice is taken orally in case of eye diseases.
Ludwigia hyssopifolia (G.Don) Exell (Onagraceae)	Herb	Leaf	Orchitis	The leaf paste (5 g) is used for poulticing in orchitis and glands in the neck for 7 days. A decoction is also used as a vermifuge and purgative.
Luffa acutangula Linn. Var. amara Roxb. (Cucurbitaceae), 'Pitataradi'	Climber	Fruit	Jaundice, asthma, leucoderma	The dried fruit powder of fruit in the form of snuff is used to cure jaundice. Fruit is also used traditionally for asthma and leucoderma.
Macrotyloma uniflorum L. Verdc. (Fabaceae), 'Kolatha'	Herb	Seed	Kidney stone	50 g of seed is soaked overnight with water. The decanted water (200 ml) is taken for thee months for removal of kidney stones.
Marsilea minuta L. (Marsileaceae), 'Sunsunia'	Herb	Leaf	Headache , blood pressure	One spoonful of leaf extract is taken with sugar for 7 days to cure headache and blood pressure.
Momordica dioica Roxb. Ex Willd. (Cucurbitaceae), 'Kankada'	Climber	Fruit	Diabetes, acidity	One spoonful of fruit juice is taken for a month to control diabetes. One glass of fruit juice with sugar candy daily for 7 days is recommended to control acidity.
Morinda citrifolia L. (Rubiaceae), 'Achu'	Tree	Root	Dysentery	Decoction of root (30-40 ml) is given for 3-5 days to cure dysentery

Botanical name, family & local name	Growth forms	Parts used	Disease/condition	Mode of application
Mucuna pruriens (L.) DC. (Fabaceae), 'Baidanka'	Climber	Seed	Ulcer	Paste of the seed is applied topically over the ulcers of the genital organs of both sexes till cure. Meat preparations are restricted in the diet during the period of treatment.
Oroxylum indicum (L.) Vent. (Bignoniaceae), 'Phanaphania'	Tree	Seed	Cough and bronchitis	One spoonful of seed (2-3 g) decoction is taken for 7 days to cure cough and bronchitis.
Ottelia alismoides (L.) Pers. (Hydrocharitaceae), 'Panikundri'	Herb	Leaf	Stomach disorder	2-5 ml leaf juice is taken for 5-7 days in treating stomach disorder.
Oxalis corniculata L. (Oxalidaceae), 'Amlitisaga'	Herb	Leaf, root	Cold	Roots are made into paste along with garlic and one teaspoonful is given twice daily for 5-7 days to cure cold. The leaves are eaten as vegetable to check burning sensation of soles.
Pandanus fascicularis Lam. (Pandanaceae), 'Kia'	Shrub	Root	Diabetes	Decoction of aerial root (2ml) is taken for 21 days to control excessive urination in diabetic patients.
Phoenix Sylvestris Roxb., (Arecaceae), 'Khajuri'	Tree	Stem	Diabetes	Fresh juice (3-5 ml) of the stem extracted early morning is taken for 5-7 days to restore potency in diabetic patients.
Pistia stratiotes L. (Araceae), 'Borajhanji'	Herb	Whole plant	Diabetes, urinary problem	The juice (10 ml) of young plant mixed with equal amount of green coconut milk is given for 30 days to reduce blood sugar. Plant decoction is taken to cure irregular urination
Sapindus emarginata Vahl. (Sapindaceae), 'Rithaphal'	Tree	Seed	Insecticide, dental carries	The powdered seeds are used as insecticide. The seeds are employed in the treatment of dental caries. It cleanses the oily secretion of skin and is even used as a cleanser for washing hair.
Smilax perfoliata Lour. (Smilacaceae), 'Ramdantuni'	Climber	Root	Gastritis, gynecology	Roots are boiled in water and this water (5 ml) is given orally for 5 days with honey to cure gastric problem like indigestion. Decoction of root is used for gynecological disorders.
Solanum viarum Dunal D.C. (Solanaceae), 'Bhegibaigana'	Herb	Fruit	Contraceptive	Burnt fruits (3-5 g) are used as oral contraceptive.
Sonneratia caseolaris L. (Sonneratiaceae), 'Orua'	Tree	Leaf	Dysentery	The decoction of leaves (3 ml) is taken for 3 days to cure dysentery.
Sphaeranthus indicus L. (Asteraceae), 'Bhuinkadamba'	Herb	Whole plant	Kidney problem	Paste of the plant (3-5 g) with a little old jiggery is taken for 5-7 days to check excessive urination.
Spinacia oleracea L. (Chenopodiaceae), 'Palanga'	Herb	Leaf, fruit	Diabetes	3 g leaf and 2-3 g fruit are fried with mustard oil and taken for 30-40 days to control frequent urination in diabetic patient.
Tephrosia purpurea (L.) Pers. (Fabaceae) , 'Banakulathi'	Shrub	Whole plant	Cardiovascular	The whole plant (3-5 g) is taken for a month to cure diseases of liver, spleen, heart and blood.
Tragia involucrata L. (Euphorbiaceae), 'Bichuati'	Herb	Root	Urinary	3-5 g root powder mixed with 50 ml curd is taken for 3-5 days to control frequent urination.
Trapa natans L. (Trapaceae), 'Pani singada'	Herb	Leaf	Rheumatism, stomach disorder	3-5 g leaf powder is taken orally for a month to get relief from rheumatism. 2 spoonfuls of leaf juice is taken for a week to regulate stomach disorder.

Botanical name, family & local name	Growth forms	Parts used	Disease/condition	Mode of application
Tribulus terrestris L. (Zygophyllaceae), 'Gokhara'	Herb	Seed	Urinary	Infusion of matured seed is taken for 7 days in empty stomach against urinary infection.
Trichosanthes cucumerina. L. (Cucurbitaceae), 'Banpotala'	Climber	Tuber	Colic	10 g tuber powder is taken orally once daily for 21 days to cure colic.
Vetiveria zizanioides (L.) Nash. (Poaceae), 'Bena'	Herb	Root	Rheumatism ,gout arthritis	1-2 teaspoonfuls of root decoction is taken for 30-40 days for the treatment of rheumatism and gout arthritis.
Vicoa indica (L.) DC. (Asteraceae), 'Banasebati'	Herb	Leaf	Stomach problem, dysentery	10 ml of leaf decoction is taken early morning for a week to cure stomach problem and dysentery.
Ziziphus oenoplia (L.) Mill. (Rhamnaceae), 'Kanakoli'	Shrub	Root	Hyperacidity, ascariasis, wounds	2 g of root paste is taken for hyperacidity, ascariasis and healing of wounds.

ISSN 2449-8947 MicroMedicine Review Article

Tooth root resorption: etiopathogenesis and classification

Marta Sak[1], Małgorzata Radecka[1], Tomasz M. Karpiński[2], Anna Wędrychowicz-Welman[1], Anna K. Szkaradkiewicz[1]

[1]Department of Conservative Dentistry and Periodontology, Poznań University of Medical Sciences, Bukowska 70, 60-812 Poznań, Poland

[2]Department of Medical Microbiology, Poznań University of Medical Sciences, Poznań, Poland

* Corresponding author: Dr hab. Anna K. Szkaradkiewicz; e-mail: aniaszk@op.pl

Received: 06 January 2016; **Revised submission:** 17 February 2016; **Accepted:** 25 February 2016

ABSTRACT

Resorption is the combination of physiological or pathological factors leading to the loss of enamel, dentin, cementum, and the alveolar bone by the action of polynuclear giant cells. There are various theories about the causes of resorption. They can be divided into local and systemic. For local reasons mainly are mentioned injuries, pulp necrosis, arising as a result of local caries, chronic pulp inflammation and iatrogenic factors, especially high temperature triggered during the cavity preparation or tooth crown preparation in prosthetic reconstruction. Depending on location of the lesion, resorption is divided into internal and external. In some cases, the two types of resorption may occur simultaneously. Untreated resorption usually leads to loss of the tooth.

Keywords: Root resorption; Internal resorption; External resorption; Ankylosis; Tooth resorption treatment.

1. INTRODUCTION

Resorption is the combination of physiological or pathological factors leading to the loss of enamel, dentin, cementum, and the alveolar bone by the action of polynuclear giant cells. These cells, depending on tissue resorbed by them are called osteoclasts, cementoclasts or dentinoclasts. Resorption is a physiological process only in milk teeth in the period preceding the teeth replacement. Pathological process however occur in both the deciduous and permanent dentition [1, 2].

Root cementum from the side of periodontium is coated with osseomucoid (precementum) and cementoblasts that protect it from resorption. A similar protective role in the dentine plays odontoblasts layer and predentin. Damage to these layers and the appearance of local inflammation predisposes to the occurrence of resorption [3, 4].

According to Fuss [5] the occurrence of resorption is conditioned by mechanical or chemical damage of tissues and further stimulation by infection or pressure. The dynamics of this process is different. After periods of increased destruction of the tooth by clastic cells is observed the process of tissue regeneration by the blast cells. These processes are superimposed on each other but in effect untreated resorption usually leads to tooth loss [6].

2. ETIOPATHOGENESIS

There are various theories about the causes of resorption. They can be divided into local and systemic. For local reasons mainly are mentioned

injuries, pulp necrosis, arising as a result of local caries, chronic pulp inflammation and iatrogenic factors, especially high temperature triggered during the cavity preparation or tooth crown preparation in prosthetic reconstruction [7]. In the literature, attention is also drawn on the influence of calcium hydroxide preparations during the biological pulp treatment (vital amputation) on resorption process [8, 9]. For systemic reasons mainly are included hypoparathyroidism, hypertension and genetic disorders.

Table 1. Causes of tooth resorption [6, 8, 10-12].

Local reasons	Systemic reasons
1. Sharp injuries	1. Hormonal disorders, e.g. hyperthyroidism and hypoparathyroidism
2. Chronic injuries	2. Hypertension
3. Microtrauma eg. related to the profession (eg. trumpeter, seamstress), crack a tooth crown	3. Atherosclerosis
4. Damage of periodontium after teeth replantation	4. Paget's disease
5. Malocclusion	5. Papillon-Lefevre syndrome
6. Improper habits	6. Stevens-Johnson syndrome
7. Too much force in orthodontic treatment	7. Hepatic impairment
8. Teeth whitening	8. Kidney disease
9. Biological treatment with calcium hydroxide	9. Bone dysplasia
10. Delayed or irregular eruption of permanent teeth	10. Genetic disorders
11. The pressure of the tumor or cyst	11. Vitamin A deficiency
12. Iatrogenic action, e.g. thermal pulp damage	12. Pregnancy
13. Dental abnormalities, e.g. invaginated teeth	13. Shingles
14. Periodontitis, incorrect execution of periodontal treatment (scaling, rootplaning)	14. Gancher's disease
	15. Turner's syndrome
15. Different dental materials (eg. silver nitrate, silicate cements)	16. Kabuki syndrome (the presence of a number of external root resorptions of the lower central incisors and molars)
	17. Radiotherapy

3. CLASSIFICATION OF TOOTH RESORPTIONS

The division of resorption is based on the location, the mechanism of formation and type of the disease process. In addition to the physiological resorption concerning milk teeth is also identified the pathological resorption, which may affect the milk and permanent teeth. The disease process may start with a tooth cavity or on the cementum surface. Depending on location of the lesion, resorption is divided into internal and external (Fig. 1). In some cases, the two types of resorption may occur simultaneously [6, 13].

4. INTERNAL ROOT RESORPTION

Internal resorption is also called the internal granuloma, internal progressive resorption, internal middle resorption, pulpoma or „pink tooth". Always it starts in the dentine (from the side of the tooth cavity) and spreads in the direction of cementum. It occurs less frequently than the external resorption. Internal root resorption is rare in permanent teeth. Usually it affects the individual anterior teeth, occasionally is observed simultaneously in several teeth. Most frequently this process is found in the teeth with not finished development, in which a layer of dentin is thin, and tubules within it arem broad. Such a structure makes them more susceptible to any pathological processes [14, 15].

A necessary condition for development of bone resorption is the presence of living cells. The pulp in the apical part of the root until the resorption place can remain alive and pathologically unchanged, whereas in the resorption cavity and tooth chamber indicates the presence of necrotic masses or pulp in the chronic inflammatory condition [6]. The process of resorption is stopped

when the entire pulp dies.

To the main causes of internal resorption include:

- chronic inflammation of the pulp on the caries process background,
- iatrogenic stimuli (eg. high temperatures triggered during cavity preparation, or prosthetic reconstruction preparation),
- acute mechanical injury,
- biological treatment using the calcium hydroxide formulations (resorption usually occurs within the first twelve months after treatment).

Internal resorption due to the location, according to Jeanneret [16] is divided into:

- Type A (intracoronal resorption),
- Type B (intra-root resorption),
- Type C (resorption with perforation of the canal wall).

Kless and Philppart distinguish the internal resorption D (perforating the wall of a tooth crown) [6, 17]. Considering the pathogenesis of the disease, internal resorption is divided into:

- inflammatory (type A, B, C, D according to [18]),
- replacement.

4.1. Internal inflammatory resorption

Internal inflammatory resorption is characterized by the rapid enlargement of the tooth cavity as a result of the ongoing pathological process. Resorption cavity can be placed both in the tooth crown (type A, D) and root (type B, C) (Figs. 1-5) [6].

Internal root resorption is usually asymptomatic and is first recognized clinically through routine radiographs. It is estimated that only in 2% of cases may occur clinical symptoms [15]. The results of tests on the pulp viability often do not deviate from the norm. They may be negative in the case of the canal perforation ongoing with the pulp necrosis. Rarely occur the exacerbation of inflammation and pain symptoms. In the advanced case of type A resorption in clinical examination of the patient can be see the pink color of the tooth crown (pink tooth), which is evidence of a very large local destruction of dentin and luminous dispersion through a thin layer of enamel granulation tissue. In the case of very extensive change the main clinical symptom is the root fracture [4]. However, most often the resorption is diagnosed accidentally on the basis of X-ray. Many studies prove the legitimacy of the conical computed tomography use for accurate diagnosis [19]. The use of CBCT allows to evaluate the nature of resorption and its exact location [20].

In the type B resorption is seen bubble or oval, symmetrical widening of the root canal with a clearly regular and well limited smooth walls. The resorption loss is uniformly saturated and flows into the tooth cavity. Characteristic is also fixed position of the change regardless of the X-ray projection [6].

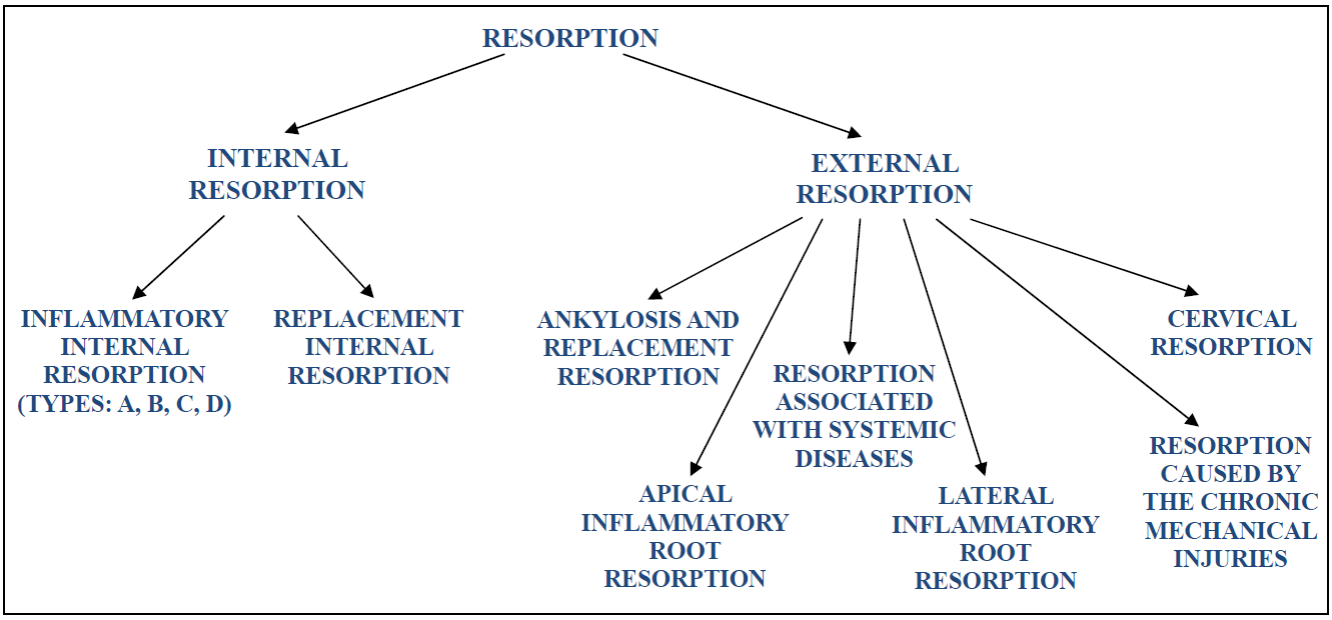

Figure 1. The classification of internal and external resorption.

In the case of the canal wall perforation (type C) additionally occurs thinning of the alveolar bone structure in the vicinity of the perforation. In recognition of this type of resorption is useful examination with endometer. Active electrode inserted into the canal lumen immediately indicates the apical foramen, giving a false result.

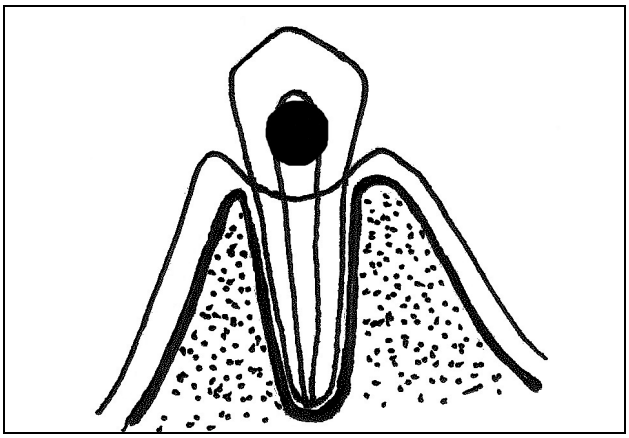

Figure 1. Internal resorption type A.

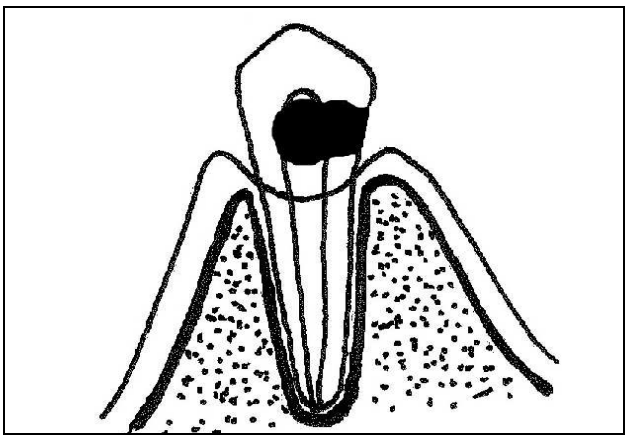

Figure 2. Internal resorption type D.

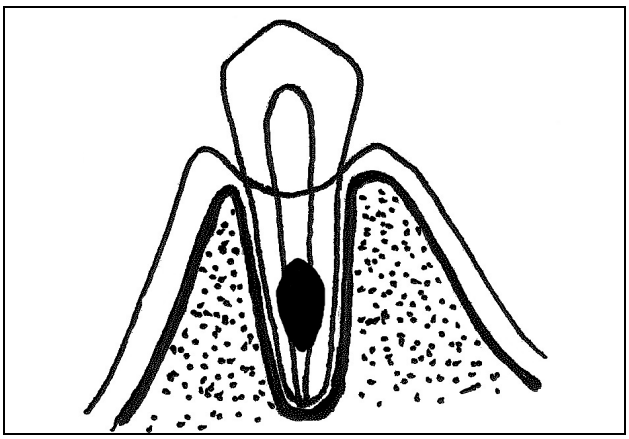

Figure 3. Internal resorption type B.

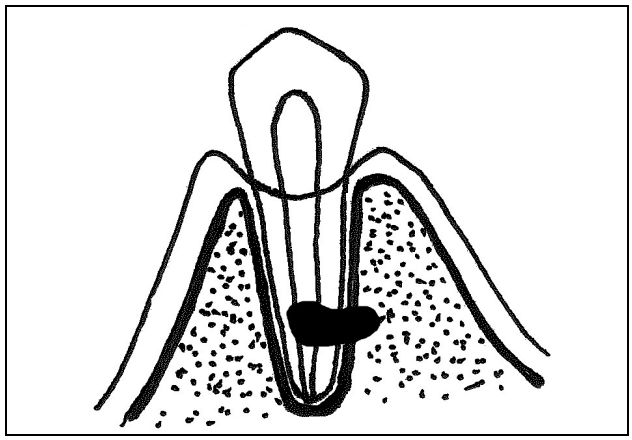

Figure 4. Internal resorption type C.

The treatment plan should be individualized, taking into account factors related to the patient, clinical and radiological features. If the tooth is not suitable for restoration, should be discarded and supplemented with prosthetic reconstruction. In the case of a good prognosis should be use conservative, surgical or conservative-surgical treatement. Each type of internal resorption due to irreversible inflammation of the pulp requires endodontic treatment in order to stop the process of root resorption and obtaining sealed reconstruction [7]. In most cases, due to the characteristic shape of the tooth cavity, it is possible to removal the contents only by mechanical preparation of the chamber and root canal. Facilitate extending in these cases is performed wider than usual in order to create a good access to the resorption cavity. In fear of iatrogenic perforation of the thinning canal walls is not recommended the preparation with machine tools. However, some authors use such instruments in treatment of their clinical cases [19]. In a canal rinse are used 5.25% sodium hypochlorite solution and ultrasounds to allow precise removal of granulation masses from resorption cavity sinuses. It is advisable the use of 3% hydrogen peroxide [17].

If unsure as to the complete removal of granulation tissue from the root canal is recommended to temporarily fill it with no-hardening preparations of calcium hydroxide for a period of several days to several weeks [8, 11]. These preparations aimed at dissolving the remaining granulation tissue. For the final filling of canal, due to the existence of post-resorption cavities are recommended thermal methods (e.g. Obtura, Thermafil). Among sealants are most recommended materials

resistant to high temperature, which strengthen the root walls, which are thinning in a result of resorption. These include materials based on epoxy resins and glass-ionomer cements [8, 21]. Some authors recommend the use of MTA material or biodentine to fill the resorption cavity. The remaining part of the canal is filled with thermal methods [11, 19, 22]. It is very important for exact cleaning and filling the canal, because inaccurate removal of granulation tissue results in the further development of pathological resorption.

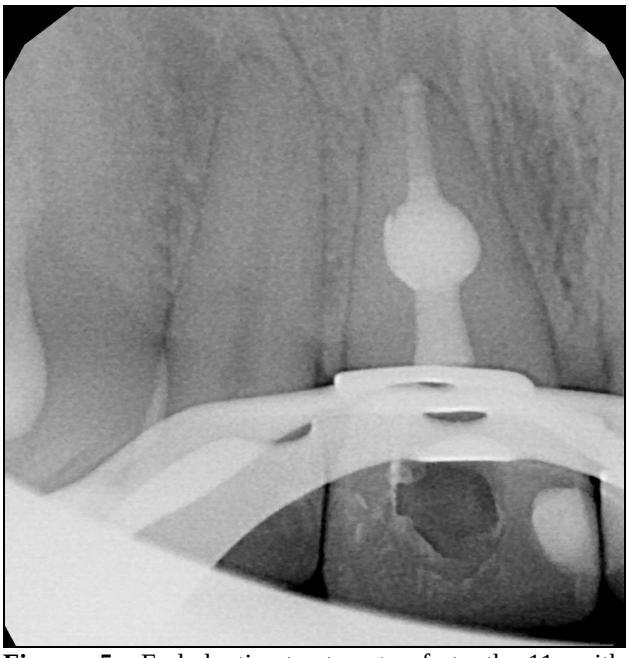

Figure 5. Endodontic treatment of tooth 11 with resorption type B.

Type A resorption is easy to treat. In the case of minor changes the dentin loss is filled with glass-ionomer cement. The outer layer of filling is made from composite material. With considerable damage of the crown threatening to her breakage is advisable to prosthetic supply of the tooth with crown-root inlay and prosthetic crown.

Treatment of resorption type C is a very difficult. The most important in the treatment is tight close of the perforations. In the case of small size resorption, without any contact with the environment of the oral cavity, procedure is the same as in type B, except that the formulations of calcium hydroxide are used for several or over a dozen months, until the formation of the barrier from the hard tissue in the place of perforation. Therapeutic procedure may be reduced to one or two visits using MTA material and closing the place of perforation on the canal side. In cases in which the endodontic therapy is unsuccessful or impossible, are used conservative-surgical methods. Place of the perforation is closed using a surgical access with MTA material. Other methods of surgical treatment are: apicectomy (if the perforation is located in the apical one third of the root), radectomy, hemisection, intended replantation or extraction [6, 14]. The effect of untreated internal resorption may be spontaneous fracture of the tooth crown or root, most frequently leading to extraction.

4.2. Replacement resorption

The causes of replacement resorption are probably mild irritations, e.g. irreversible chronic inflammation of the pulp or weak injury. This change is characterized by resorption of dentin with simultaneous deposition of hard tissues resembling bone or cementum in the tooth cavity. On the x-ray is visible widening of the tooth cavity and deposition of the loose tooth structures in the light. This can be cause of partial obliteration of canals or (in the long progressive changes) total obliteration of the chamber and root canal [1].

5. EXTERNAL ROOT RESORPTION

The immediate cause of pathological external resorption of the cementum is damage of cementoblasts and cementoid by pathogenic stimulus, which disturbs the balance between the activities of cementoblasts and osteoclasts. In the progressive process resorption involves the entire thickness of the dentine and cementum, causing outside perforation of the canal. It is also characteristic process in the adjacent alveolar bone. Minor irritants can cause the transient resorption, reversible after eliminating the causative stimulus [14].

In the literature there is not uniform division of external resorption. Because the major etiological factor and the pathogenesis are distinguished:

1. apical inflammatory root resorption accompanying chronic apical inflammations,
2. lateral inflammatory root resorption accompanying post-traumatic necrosis of the pulp,
3. cervical resorption,

4. ankylosis and replacement ankylosis resorption, osseous replacement,
5. resorption caused by the action of chronic mechanical trauma,
6. resorption associated with systemic diseases.

External resorption due to the location is divided into:

1. apical resorption
2. resorption of the central part of the root
3. cervical resorption [6].

5.1. External inflammatory resorption

External inflammatory resorption is the most common type of external resorption (Figs. 6-8). It is characterized by deep, bowl-shaped depressions in the cementum and root dentine. Mostly it concerns the root apex, less likely its side surface. The development of external resorption usually occurs when the infection overlaps the injury. Etiological agent may also be endodontic, periodontal and orthodontic treatment or pressure of impacted tooth for the root of adjacent tooth.

Apical root resorption is most common in the purulent inflammations and cysts, less often in granulomas. On the other hand lateral inflammatory root resorption is observed in the teeth with dead, infected pulp or after endodontic treatment.

Clinically very important is the fact that the osteoclasts colonize and resorbe root tissues only in the presence of a stimulating agent. His absence causes inhibition of this process, and cavities are filled with osteoblasts reconstructed lost tissues.

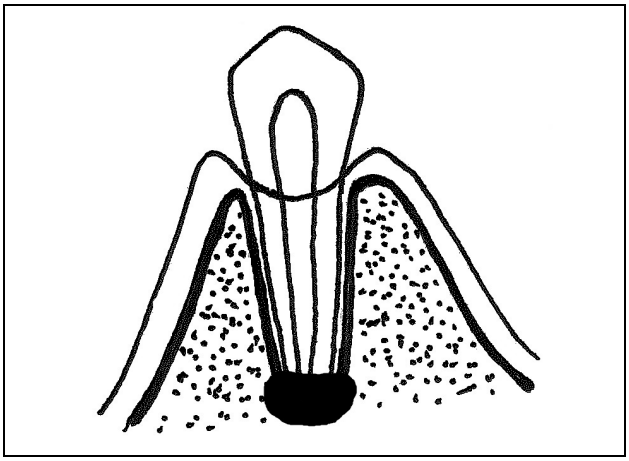

Figure 6. Apical root resorption.

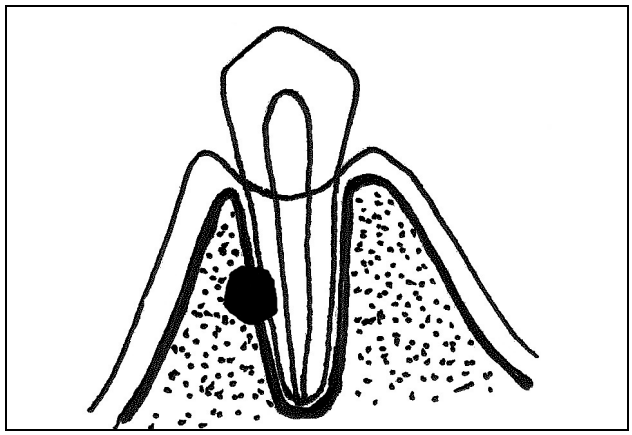

Figure 7. Lateral root resorption.

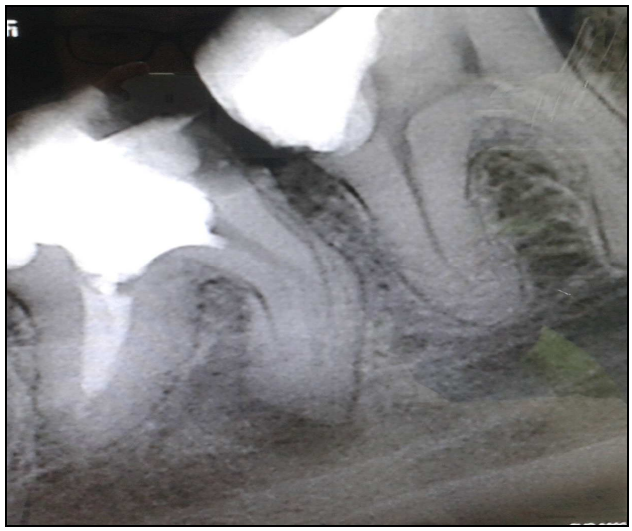

Figure 8. External apical resorption of the proximal root of the tooth 47.

In the X-ray image in both types of resorption is visible loss of hard tissues, both in the root and in the surrounding bone. The shape of the cavity is irregular with a faint outline. It is located asymmetrically with respect to the tooth midline. Resorption area can "impose" on the course of the canal, which always remains visible and undeformed [23, 24].

Treatment of small resorptions consists of to determine the cause of the disease and its elimination. Minor irritants such as trauma or a history of orthodontic treatment can cause transient type of surface resorption. Teeth do not give then any discomfort, the pulp usually remains alive. In the X-ray is found a small loss of tooth tissues, which leads to rounding the apex. The bone and compacted alveolar plate look right [7]. Treatment consists of eliminating the causative factor. In turn,

the progression of changes seen on control radiographs is an indication to initiate endodontic treatment. Treatment in these cases is during several sessions and relies on temporary filling of canals with calcium hydroxide or antibiotic-steroid preparations. Once the disinfection is obtained canal should be filled [6]. In the case of the wide apical foramen as a result of the ongoing resorption process it is recommended to supply this place with MTA material on the min. 3 mm [25]. Extensive resorptions of permanent teeth are indications to resection, amputation or root hemisection, and in some cases, to extraction of the tooth.

5.2. Cervical resorption

Cervical resorption (Fig. 9) is quite often occurring phenomenon, appearing always in the place of epithelial attachment damage and formation of the periodontal pocket. The etiology of this process is not fully understood. The most common factors that cause this type of resorption are: intracavity teeth whitening, damage of the cementum surface due to tooth trauma, improperly performed periodontal procedures (scaling) or orthodontic treatment. Often, for unknown reasons, may appear several years after the injury action. In the course of this disease process there is resorption of cementum, enamel and dentin, and in the final stage of resorption may also include pulp. In connection with such a way of resorption spreading inside the root, cervical resorption it is also known as invasive [26].

Cervical external resorption is usually asymptomatic. Most often it is detected incidentally during radiological examination. Root resorption is always accompanied by the loss of adjacent alveolar bone, giving the radiological image of subalveolar bone pocket [23, 27]. This form of resorption spreads generally in the direction of the root, but it rarely leads to perforation of the canal wall. In a significant destruction of dentine around the tooth neck during the examination of the patient is observed the pink spot in the crown of the tooth.

The success of the treatment of cervical inflammatory resorption is conditioned primarily by disease diagnosis in its early stage. If the change is limited, and access to resorption cavity is good, it is possible to decide on surgery. If the tooth is alive, and during surgery could result in the exposure of the pulp, it should be first performed endodontic treatment. Traditional methods of procedure depend on surgical exposure of the lesion site, removing of resorption granulation by curettage and filling of the root loss with composite, glass-ionomer cement, compomer or MTA [28]. An alternative method of treatment is to eliminate the resorbing tissue by chemical means. Used for this purpose is a 90% aqueous solution of trichloroacetic acid. Depending on the amount of resorption tissue acid is applied for 2-4 minutes. After this time, dead tissue should be remove and filling of the resorption cavity should be begin [26].

Figure 9. Advanced cervical resorption of 12 tooth after intracavity bleaching.

5.3. External replacement resorption and ankylosis

Dentoalveolar ankylosis is characterized by a combination of the root structure of the tooth directly with bone tissue, while replacement resorption is the process of the loss of root tissues and replacing them through the bone [14, 29]. As the cause of ankylosis and replacement resorption most frequently are mentioned injuries, including tooth dislocation, axial and lateral intrusion, as well inflammatory infection of the dead pulp, pressure, disorder in the local blood circulation, genetic factors, vitamin A deficiency and hormonal imbalance.

Dentoalveolar ankylosis (Fig. 10) and replacement resorption (Figs. 11-14) are most common in

young people (8-16 years old). Their origin is connected with damage of cementum and periodontal fibers. This process may be reversible, when applies less than 20% of the root surface [30]. The earliest post-traumatic ankylosis can be recognized after approx. 2 months after the onset of the injury.

Symptoms of both resorption types are: no physiological mobility of the tooth, metallic flicking sound and radiologically absence of periodontal slit and in advanced form of resorption characteristic is X-ray image of "root eaten by moths." In extremely severe cases can occur almost complete replacement of cementum and root dentin by bone tissue until completely blurring of the tooth outline in X-ray. In the initial stage of changes intraoral X-ray can not demonstrate any deviations from the norm, because ankylosis develops on the lingual and labial sites. In such cases, it is useful execution of CBCT. In young patients ankylosis prevents physiological mesial movement of teeth and inhibits the local growth of alveolar bone, which is sinking into the surrounding alveolar ridge – infraposition.

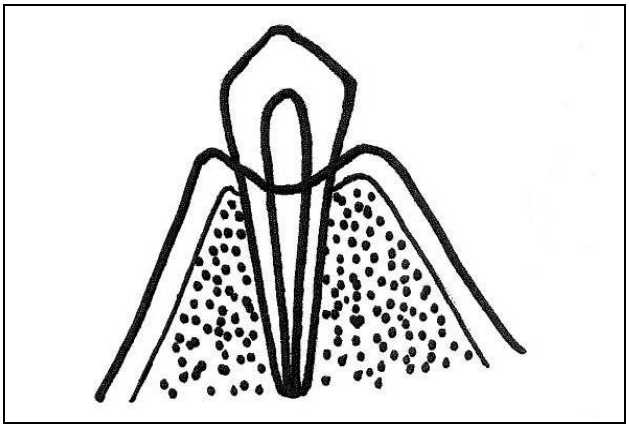

Figure 10. Ankylosis.

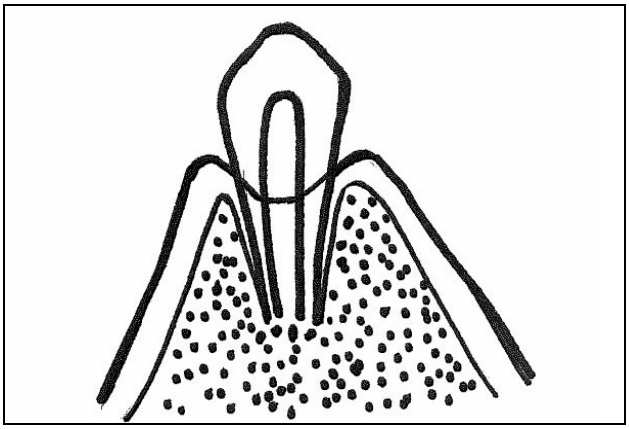

Figure 11. Replacement resorption.

Figure 12. Replacement resorption of the tooth 24.

Figure 13. Replacement resorption of the tooth 46.

Figure 14. Replacement resorption of mesial root of the tooth 36.

Ankylosis and replacement resorption are the only types of resorption that fail to stop using endodontic treatment and tooth loss is inevitable and depends on the rate of bone turnover. It is

now believed that the most effective treatment of replacement resorption in young people in the period of growth is decrowning [31, 32]. This procedure consists in cutting off the tooth crown 1.5-2 mm below the edge of the bone and leaving the root, which in the result of replacement resorption will be replaced by bone. This method is intended to prevent the loss of alveolar bone, stimulate its growth and provide optimal conditions for future prosthetic reconstruction. After about 18 years of age, when will be completed the growth process, even at partial replacement of the tooth root by bone, is possible to perform the implantation.

In older patients it is acceptable to leave the tooth affected by resorption to the appearance of the symptoms of inflammation. In the case of the bite disturbances with severe crowding of the teeth and the unfavorable profile it is acceptable as possible atraumatic removal of such tooth and making autologous premolar transplantation. This procedure results from the necessity of the extraction treatment of the malocclusion. In cases of punched teeth stored for a long time in a dry environment resorption can be delayed (but not stopped!), if the root surface is coated with a layer of fluoride before the replantation [6, 33, 34].

5.4. Resorptions caused by the action of chronic mechanical trauma

Chronic mechanical injuries are the result of pressure by the unerupted teeth or improperly erupted, expanding cysts, tumors and as a result of points of premature occlusal contacts. These factors can cause resorption of the roots of adjacent teeth. Resorption initially causes little defects located in the site of force action, but in more advanced cases also relates to the dentine, leading to a root reduction or even its total destruction. External resorption of the teeth roots is also often a consequence of orthodontic treatment. The location and extent of the post-resorption defects depend on the direction, time of action and size of the force applied in orthodontic apparatus. On the X-ray is found a widening of periodontium space and shorten of the roots that appear like cut off. Treatment of this resorption type is to eliminate the causative agent. During orthodontic treatment for prophylaxis are used by the patient interruptions in wear a fixed apparatus, which is aimed at inhibiting resorption, along with the reconstruction of hard tissues of the tooth [2].

5.5. Resorptions associated with systemic diseases

Internal and external resorptions may be accompanied by some systemic diseases. For these include: hyperparathyroidism and hypoparathyroidism, hyperthyroidism, Paget's disease, Gaucher's disease, Turner syndrome, cancers within the facial part of the skull. Frequently these resorptions concern several teeth and are chronic [2].

6. CONCLUSIONS

Correct diagnosis is crucial in the selection of the treatment plan. It is known fact that the internal and external resorption have different etiology and require different therapeutic protocols. Appropriate therapy increases the chances of maintaining the tooth in the mouth, which is particularly important in the esthetic zone of the patient.

AUTHORS' CONTRIBUTION

MS, MR, TMK and AKS: manuscript preparation; MR: pictures; AWW and AKS: collection of references, manuscript corrections; TMK: final editing and checking of manuscript. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

REFERENCES

1. Barańska-Gachowska M. Endodontics developmental and mature age [in Polish]. Czelej, Lublin 2004.
2. Prażmo E, Mielczarek A. Current viewpoints on the etiology and treatment of tooth resorptions. Nowa Stomatol. 2014; 1: 53-58.
3. Jurczak A, Kołodziej I, Kościelniak D, Słowik J. External replacement root resorption as a late posttraumatical complication in young patients [in Polish]. Implantoprotetyka. 2009; 10(4): 37.

4. Perlea P, Nistor CC, Suciu I, Iliescu MG, Iliescu AA. Rare multiple internal root resorption associated with perforation - a case report. Rom J Morphol Embryol. 2014; 55(4): 1477-1481.
5. Fuss Z, Tsesis I, Lin S. Root resorption - diagnosis, classification and treatment choices based on stimulation factors. Dent Traumatol. 2003; 19(4): 175-82.
6. Arabska-Przedpełska B, Pawlicka H. Modern endodontics in practice [in Polish]. Bestom, Łódź 2011.
7. Al-Momani Z, Nixon PJ. Internal and external root resorption: aetiology, diagnosis and treatment options. Dent Update. 2013; 40: 102-104, 107-108, 111-112.
8. Ciesielski P, Łaszkiewicz J. Internal inflammatory root resorption - on the basis of literature and own experience. J Stomatol. 2008; 61(1): 40-47.
9. Bastos JV, de Souza Cortes MI, Goulart EMA, Colosimo EA, Gomez RS, Dutra WO. Age and timing of pulp extirpation as major factors associated with inflammatory root resorption in replanted permanent teeth. J Endod. 2014; 40(3): 366-371.
10. Szyszkowska A, Jachewicz T. Teeth resorption. Division, origin and microscopic picture [in Polish]. Implantoprotetyka. 2010; 11(1): 38.
11. Yadav P, Rao Y, Jain A, Relhan N, Gupta S. Treatment of internal resorption with mineral trioxide aggregates: a case report. J Clin Diagn Res. 2013; 7(10): 2400-2401.
12. Cholia SS, Wilson PHR, Makdissi J. Multiple idiopatic external apical root resorption: report of four cases. Dentomaxillofac Radiol. 2005; 34(4): 240-246.
13. Andreasen JO. Luxation of permanent teeth due to trauma. A clinical and radiographic follow-up study of 189 injured teeth. Eur J Oral Sci. 1970; 78(1-4): 273-286.
14. Hargreaves KM, Cohen S. Cohen's pathways of the pulp. 10th ed. Mosby Elsevier, 2011: 639-647.
15. Thomas P, Pillai RK, Ramakrishnan BP, Palani J. An insight into internal resorption. ISRN Dentistry. 2014: ID 759326.
16. Jeanneret R. Beitrag zum sog. „internen Granulom". Schweiz Monatsschr. Zahnheilkd. 1947; 57: 378-382.
17. Lipski M, Woźniak K, Radlińska J, Łagocka R. Endodontic treatment of teeth with internal resorption. Case descriptions. Mag Stomatol. 2002, 10: 42-46.
18. Ne RF, Witherspoon DE, Gutmann JL. Tooth resorption. Quintessence Int. 1999; 30(1): 9-25.
19. Esnaashari E, Pezeshkfar A, Fazlyab M. Non-surgical management of an extensive perfortative internal root resorption with calcium-enriched mixture cement. Iran Endod J. 2015; 10(1): 75-78.
20. Nance RS, Tyndall D, Levin LG, Trope M. Diagnosis of external root resorption using TACT (tuned-aperture computed tomography). Dental Traumatol. 2000; 16(1): 24-28.
21. Goldberg F, Massone EJ, Esmoris M, Alfie D. Comparison of diffrent techniques for obturating experimental internal resorption cavities. Endod Dent Traumatol. 2000; 16(3): 116-121.
22. Ebeleseder KA, Kqiku L. Arrest and calcification repair of internal root resortion with a novel treatment approach: report of two cases. Dent Traumatol. 2015; 31(4): 332-337.
23. Patel S, Kanagasingam S, Pitt Ford T. External cervical resorption: a review. J Endod. 2009; 35(5): 616-625.
24. Patel S, Ricucci D, Durak C, Tay F. Internal root resorption: a review. J Endod. 2010; 36(7): 1107-1121.
25. Mittal S, Kumar T, Mittal S, Sharma J. Internal root resorption: an endodontic challenge: a case series. J. Conserv Dent. 2014; 17(6): 590-593.
26. Heithersay GS. Clinical endodontic and surgical management of tooth and associated bone resorption. Int Endod J. 1985; 18(2): 72-92.
27. Kandalgaonkar SD, Gharat LA, Tupsakhare SD, Gabhane MH. Invasive cervical resorption: a review. J Int Oral Health. 2013; 5(6): 124-130.
28. Aggarwal V, Singla M. Management of inflammatory root resorption using MTA obturation - a four year follow up. Brit Dent J. 2010; 208(7): 10.
29. Trope M. Clinical management of the avulsed tooth: present strategies and future directions. Dent Traumatol. 2002; 18: 1.
30. Lindskog S, Pierce AM, Blomlof L, Hammarstrom L. The role of the necrotic periodontal membrane in cementum resorption and ankylosis. Endod Dent Traumatol. 1985; 1: 96.
31. Malmgren B, Cvek M, Lundberg M, Frykholm A. Surgical treatment of ankylosed and infrapositioned reimplanted incisors in adolescens. Scand J Dent Res. 1984; 92: 391-399.
32. Sapir S, Kalter A, Sapir MR. Decoronation of an ankylosed permanent incisor: alveolar ridge preservation and rehabilitation by an implant supported porcelain crown. Dent Traumatol. 2009;

25(3): 346-349.

33. Buczek O, Zadurska M, Osmólska-Bogucka A. Ankylosis in adolescence - treatment options with special focus on decoronation - review of literature. J Stomat. 2014; 67(3): 346-359.
34. Kowalczyk K, Wójcicka A, Iwanicka-Grzegorek E. External resorption of tooth hard tissues and alveolar bone - formation pathomechanism. Nowa Stomatol. 2011; 4: 170-174.

ISSN 2449-8947 MicroMedicine Case Report

Rare presentations of extrapulmonary tuberculosis in women

Minakshi Rohilla, Arun Kalpdev, V. Jain, G. R. V. Prasad, J. Kalra

Department of Obstetrics and Gynecology, Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, 160012 India

* Corresponding author: Dr Minakshi Rohilla, Add. Prof.; Mobile: +91-9914209354; Fax: 0172-2747909; E-mail: minurohilla@yahoo.com

Received: 17 January 2016; **Revised submission:** 28 February 2016; **Accepted:** 15 March 2016

ABSTRACT

India has high burden of tuberculosis (>60 cases per 1 lac population per year) accounting for 30% burden of the total TB cases in the world. It is a leading cause of death of women amounting to approximately 500000 deaths every year. TB is also one of the leading non obstetric causes of maternal mortality with an estimated 33% deaths in women of child bearing age. There is limited data on extrapulmonary tuberculosis (EPTB) during pregnancy and postpartum period. Pregnancy being an immune compromised state, adds the theoretical risk for getting tubercular infection in women. Due to vague and nonspecific symptomatology of EPTB the diagnosis is often missed or late in pregnancy. There is variable opinion about effects of pregnancy on tuberculosis and vice versa, mostly showing no effect on each other. Other types of EPTB like genital, meningeal and abdominal tuberculosis are rare in pregnancy and are associated with variable maternal as well as fetal outcomes. Rare presentations of extrapulmonary TB in reproductive age women like secondary amenorrhoea, tuberculosis of the cervix, tubercular meningitis and postpartum tuberculosis as puerperal sepsis, perforation peritonitis and wound sinus formation are being discussed in the present series. Prompt diagnosis and early treatment especially during pregnancy are of paramount importance for maternal health.

Keywords: Extrapulmonary tuberculosis; Women; Pregnancy.

1. INTRODUCTION

Tuberculosis (TB), mainly caused by mycobacterium tuberculosis, occurs most frequently during child bearing age in women. India has high burden of tuberculosis (>60 cases per 1 lac population per year) accounting for 30% burden of the total TB cases in the world [1]. It is a leading cause of death in women amounting to approximately 500000 deaths every year [2]. TB is also one of the leading non obstetric causes of maternal mortality with an estimated 33% deaths in women of child bearing age [3].

Extrapulmonary tuberculosis (EPTB) has become an important clinical problem in past few years. The extrapulmonary sites most commonly involve lymph nodes, pleura, genitourinary tract, bones and joints, meninges, peritoneum, and pericardium [4]. Female gender, itself, is a risk factor for development of tubercular disease. It has been studied that women are prone to develop tubercular infections and resulting tubercular disease as

compared to men [5]. Pregnancy being an immune compromised state, adds the theoretical risk for getting tubercular infection in women. Due to vague and nonspecific symptomatology of EPTB the diagnosis is often missed or late in pregnancy. Though in areas with higher prevalence of tuberculosis, like India investigation protocols includes evaluation of tuberculosis. There is limited data on extrapulmonary tuberculosis during pregnancy and postpartum period. Studies have shown that biological changes in pregnancy and postpartum period may affect tuberculosis infection [6].

Table 1. Extrapulmonary tuberculosis cases.

Case	Clinical presentation	Diagnosis	Imaging	Type of extra-pulmonary TB	Family history of tuberculosis	Treatment given	Response to ATT
1	Fever, vomiting and altered sensoruim in third trimester of pregnancy	Brain imaging and CSF analysis (low sugars and lymphocytosis)	Communicating hydrocephalus with periventricular ooze (CT scan head)	Meningitis	Yes	Systemic steroids, ATT, VP shunting	Died
2	Post cesarean fever, abdominal pain and distension (post op day 3)	Acid fast bacilli smear in ascitic fluid	Dilated gut loops with ascitic fluid in between the gut loops (Abd. USG)	Peritonitis	None	ATT	Adequate
3	Post delivery (day 22) puerperal sepsis with intestinal obstruction	X-ray, USG - dilated gut loops with air fluid levels, HPE (gut) - granulomatous inflammation	CECT - extravasation of dye suggestive of perforation	Peritonitis (intraop-perforation and multiple ileal structures)	None	Gut resection, ileostomy, ATT	Died
4	Young nulligravida with secondary amenorrhoea	Endometrial biopsy (HPE and MGIT-BACTEC culture)	Endometrium was very thin, without any adnexal mass (pelvic ultra-sonography)	Endometritis	None	ATT	Adequate
5	Perimenopausal lady with cervical growth and persistent vaginal discharge	HPE (chronic granulomatous inflammation with formation of tubercular granulomas)	none	Cervicitis	None	ATT	Adequate
6	Nonhealing post cesarean scar sinus	AFB smear and culture of sinus exudates	Sinus with small tract in the caesarean scar tissue (sinogram)	Cutaneous	None	ATT	Adequate

CSF - cerebrospinal fluid, ATT - anti tubercular treatment, AFB - Acid fast bacilli, HPE - Histopathological examination, MGIT - Mycobacteria Growth Indicator Tube, BACTEC - Becton Dickinson, Abd. - abdominal, VP - Ventriculoperitoneal.

There is variable opinion about effects of pregnancy on tuberculosis and vice versa, mostly showing no effect on each other. Other types of extrapulmonary tuberculosis like genital, meningeal and abdominal tuberculosis are rare in pregnancy and are associated with variable maternal as well as fetal outcomes. Rare presentations of extrapulmonary TB in reproductive age women like secondary amenorrhoea, tuberculosis of the cervix, tubercular meningitis and postpartum tuberculosis as puerperal sepsis and wound sinus formation are being discussed in the present series.

2. CASE REPORTS

Extrapulmonary tuberculosis cases are presented in the Table 1.

3. DISCUSSION

Tuberculosis is deadly disease that kills more women of reproductive age than all the combined causes of maternal mortality [7]. The effect of TB on women depends upon on type of tuberculosis, organ of involvement, period of gestation, immunity states and co-existence of HIV infection. Commonly prevailing poor nutritional status, anaemia, hypoproteinemia and delay in diagnosis and treatment increases maternal morbidity and mortality.

Most of the cases reported in literature were diagnosed during pregnancy with atypical presentation. Pregnancy with tubercular mastitis, peritonitis, pericarditis, peritonitis, miliary, urinary, iliocecal, gastrointestinal, CNS, osteomyelitis, endometrial, renal, lymphatic and miliary forms have been reported in literature as case reports and case series.

Tuberculosis meningitis (TBM) in pregnancy is apparently very rare complication of pregnancy. Mortality and neurological morbidity may occur more frequently during pregnancy. Less than 100 cases of tuberculosis meningitis in association with pregnancy have been described in the literature so far. Articles illustrate both the serious nature and variable presentation of TBM in pregnancy. Kutlu et al. in 2007 published a case of TBM presenting as hyperemesis gravidarum [8]. High mortality has been reported in few case series but commonly correlate with delay in diagnosis and treatment. Exacerbation of TBM during second trimester of pregnancy or first month postpartum has been suggested by some author [9]. Case 1 also presented late with TBM in pregnancy. She already had neurological injury at the time of presentation and died undelivered. She was started on ATT on high index of suspicion, treatment often needs to be started empirically and neurosurgical interventions may be lifesaving [10, 11].

The worst prognosis of TB in pregnancy has been reported in women in whom a diagnosis of advanced disease is made in the puerperium [12]. Case 2 presented as puerperal sepsis with peritonitis following full term vaginal delivery on seventh postpartum day. Tubercular peritonitis in puerperium is rare and its incidence has been reported to be less that <1% in of TB cases in Japan [12]. It is hypothesised that infection most commonly occurs following reactivation of latent tuberculosis in the peritoneum. It can also follow via haematogenous spread from active pulmonary or military TB. Rarely tubercular bacilli may enter peritoneal cavity via infected intestine or fallopian tubes [13].Case 3 presented as perforation peritonitis secondary to infected intestine. These patients commonly present with abdominal pain, fever and ascites [14]. Rarely postpartum patient with tuberculous peritonitis may mimic malignant tumour with ascites and elevated serum CA125 [12]. Atypical presentation in the form of shock in postpartum period has also been reported [15]. Our patient (case 2) presented with postpartum abdominal pain (and ascites). She had dramatic improvement after initiation of ATT. Case 3 could not be salvaged because of late diagnosis of perforation peritonitis secondary to intestinal tuberculosis. In a series of 23 patients with tuberculosis peritonitis 12 (52%) had positive ascitic fluid for AFB, all except one completed ATT success fully and cured [14].Abdominal tuberculosis should be considered in patient presenting with abdomen pain, fever and ascites. This condition carries good prognosis, if promptly diagnosed and treated.

Genital TB (GTB) represents about 15-20% of extrapulmonary tuberculosis, give rise to nearly one third cases of female infertility in India. Endometrium is affected with genital TB in about 50-60% cases, however 20% cases of GTB only, presents with menstrual disturbance. Only 2.5% cases of genital TB may present with total destruc-

tion of endometrium with subsequent secondary amenorrhoea and asherman's syndrome. Case 4 presented as young married female with amenorrhoea and asherman syndrome. Conception rates have been reported to be low around (19%) with live birth rate around 7% only [16].

Tubercular cervicitis, as seen in case 5, constitutes about 0.1-0.65% of all cases of TB. Tuberculosis cervix rarely presents as growth on the Cervix, mimicking cervical cancer. Case 5 also presented as peri menopausal lady with cervical growth and later diagnosed as tuberculosis of cervix on histopathological examination (HPE) of cervical biopsy. She was cured with 6 months of ATT. Hence a high index of suspicion of TB is required in asymptomatic female presenting with abnormal cervical examination or smear especially in high burdened tuberculosis country like India [17].

Wound sinus is a connecting tract between epidermis and underlying subcutaneous tissue. Sinus may be the result of an underlying infection, an abscess formation and in the presence of a foreign body. Surgical wound infection with mycobacterium is very rare. Case 6 presented as discharging wound sinus in a caesarean scar that did not respond to antibiotics. Caesarean sections are performed worldwide for varied indications. Tuberculous sinus formation in a caesarean section scar is extremely rare however; same has been reported after hysterectomy and bone surgery [18].

Extrapulmonary tuberculosis presents at almost every site of body. In most case series the order of organ involvement are lymph nodes, pleural, genitourinary, bones and joints, meninges, bowel and peritoneum, pericardium and the skin. Women living with HIV are at higher risk of tuberculosis, so HIV testing is recommended if a diagnosis of extrapulmonary tuberculosis is considered. All six cases presented here were HIV negative and had uncommon sites of organ involvement. Two women expired due to advance disease already established in pregnancy. Because of feminism of HIV epidemic, tuberculosis in women will be seen more frequently in both developed as well as developing country. Improved diagnosis and treatment in women of reproductive age especially during pregnancy are keys of successful perinatal outcome. All preventive, diagnostic and therapeutic modalities of tuberculosis are cost effective hence should be integrated in to routine maternal health services in high burdened country like India.

AUTHORS' CONTRIBUTION

MR: Conception and design, acquisition of data, writing, review and revision of the manuscript; AK: Writing of the manuscript; VJ: Administrative and material support; GRVP: Administrative and material support; JK: Administrative and material support. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

REFERENCES

1. Mathad JS, Gupta A. Tuberculosis in pregnant and postpartum women: epidemology, management and research gaps. Clin Infect Dis. 2012; 55(11): 1532-1549.
2. Miyani C, Meintyre J. Tuberculosis in pregnancy. BJOG. 2011; 118: 226-231.
3. Arora VK, Gupta R. Tuberculosis and pregnancy. Ind J Tub. 2003; 50: 13-16.
4. Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, eds. Mycobacterial diseases. Harrison's principles of internal medicine. 18th edn. The McGraw-Hill Companies, 2011.
5. Hayward AC, Watson JM. Tuberculosis in England and Wales 1982-1993: notifications exceeded predictions. Commun Dis Rep CDR Rev. 1995; 5: R29-R33.
6. Zenner D, Knujshaar ME, Andrew N, Abubakar I. Risk of tuberculosis in pregnancy: a national primary care based cohort and self-controlled case series study. Am J Respir Crit Care Med. 2012; 185: 779-784.
7. Jana N, Vasishta K, Saha SC, Ghosh K. Obstetrical outcomes among women with extrapulmonary tuberculosis. N Engl J Med. 1999; 341: 645-649.
8. Kutlu T, Tugnu S, Aydin A, Oral O. Tuberculosis meningitis in pregnancy presenting as hyperemesis gravidarum. J Maternal Fetal Neonat Med. 2007; 20(4): 357-359.
9. Prévost MR, Fung KM, Fung K. Tuberculous meningitis in pregnancy - implications for mother and fetus: case report and literature review. J Maternal Fetal Neonat Med. 1999; 8(6): 289-294.

10. Kingdom JCP, Kennedy DH. Tuberculous meningitis in pregnancy. Int J Obstet Gynaecol. 1996: 233-235.
11. Samson EI, Gomerep S, Johnson M, Basil BA. Delayed diagnosis of tuberculous meningitis in a pregnant Nigerian: a case report. 2013; 2(1): 54-57.
12. Hiroyo I, Akika YMI, Makoto E, Hirokazu Y, Tatsuya N, Katsukcko Y, et al. Postpartum tuberculous peritonitis mimicking malignant tumour with ascites and elevated serum 125. Adv Obstet Gynaecol. 2004; 56(1): 5-9.
13. Tang LC, Cho KH, Wong Taam VC. A typical presentation of female genital tract tuberculosis. Eur J Obstet Gynaecol Repod Gol. 1984; 17: 355.
14. Piyrazoglu OK, Timurkaan M, Yalniz M, Ataseven H, Bogukan M, Bachcecioglu IH. Clinical review of 23 patients with tuberculous peritonitis: presenting feature and diagnosis. J Dig Dis. 2008; 9(3): 170-174.
15. Monika A, Ananja D, Santer A. Pelvic tuberculosis and shock in the puerperium. 2011; 104(5): 358-359.
16. Nabag WOM, Hassan AN, Sayed DEM, El-Sheikh MA. Endometrial tuberculosis and secondary amenorrhea: a report of three cases in Sudan. J Min Invas Surg Sci. 2012; 1(1): 30-33.
17. Kalyani R, Shella S, Rajinim. Cytological diagnosis of tuberculosis cervicitis: a case report with review of literature. J Cytol. 2012; 29(1): 86-88.
18. Begum HA. Case reports post operative tuberculous wound infection: A report of 6 cases. J Dhaka National Med Coll Hos. 2011; 17(2): 49-51.

ISSN 2449-8947 MicroMedicine Research Article

Cutaneous leishmaniasis in Al-Sawalha area, Lahj governorate, Yemen

Nagat Ali Muqbil[1]*, Adelah Mohammed Qaid Muqbel[2]

[1] Faculty of Science, Biology Department, Aden University, Yemen
[2] Faculty of Education, Biology Department, Aden University, Yemen
* Corresponding author: Nagat Ali Muqbil; Phone: 009672 366321; Mobile: 00967 733341311;
E-mail: Nagat_a_muqbil@hotmail.com

Received: 23 January 2016; **Revised submission:** 14 March 2016; **Accepted:** 30 March 2016

ABSTRACT

Cutaneous leishmaniasis (CL) is a serious health problem in Yemen. Although there are some previous reports, but it was not fully documented in all areas of Yemen. Alswalaha area is one of the areas in Yemen, which is not documented. Therefore the current study aimed to determine the prevalence of CL in ten villages in this studied area during the period from July 2011 - March 2012. Stained with Giemsa skin slit smears revealed amastigotes in 75 cases (91%) out of 82 examined individuals. The positive cases were confirmed by culture in NNN medium. Infections were detected in both sexes. Males are often infected (60%) than females (40%). Lesions were recorded in different age groups, with the largest number of patients (38 cases) in the age group 10-20 years. A dry skin type of leishmaniasis due to *L. tropica* is more prevalence (65%) in the studied area than the wet type (35%). The most ulcers were recorded on the face of 40 patients (45%), in the upper extremity of 25 cases (29%), and in the lower extremity of 17 cases (20%). Size of the lesions ranging between 3 mm to 5 cm. Most of the patients (73%) have single lesion, 20% of patients have two lesions, while 3 and more lesions developed 7% of patients. The duration of the infection varied from one month to more than a year. The studies conclude that Al-Swalaha is one of the endemic areas in Yemen that in need of high attention of authorities and the society.

Keywords: Cutaneous leishmaniasis; *Leishmania tropica*; Endemic; Yemen.

1. INTRODUCTION

Leishmaniasis is one of the major vector borne communicable diseases of the world [1]. It is a zoonotic infection that is caused by obligate intracellular protozoa of the genus *Leishmania*. Natural transmission of *Leishmania* parasites is carried out by sand flies of the genus *Phlebotomus* (Old World) or *Lutzomyia* (New World) [2]. There are over 20 species of leishmania that have been recorded as causative infected agents to human, the distribution of each is determined by distribution of vector, reservoir host or both [3, 4]. *Leishmania* parasites cause three forms of leishmaniases according to the localization of the parasites in mammalian tissues, these are visceral, cutaneous, and mucosal leishmaniasis. The outcome of infection depends on the species of *Leishmania* and the host's immune responses [5]. On a global scale, approximately 350 million people live in areas of active

transmission of *Leishmania*, with 14 million people throughout Africa, Asia, Europe, and the Americas directly affected by this disease [6]. The global burden of leishmaniasis has remained stable for some years, causing morbidity and mortality loss of 2.4 million disability adjusted life-years and approximately 70,000 deaths, a significantly high rank among communicable diseases [7, 8]. CL due to *L. tropica* was classically considered to be anthroponotic, zoonotic, it has been reported in many areas in middle east Saudi Arabia, Afghanistan, Iran, Turkey, Syria, Palestine, Jordan and Israel, where they caused extensive morbidity [9-12]. CL seems to be endemic in north-western Yemen, it was previously identified in Dhamran region in Taiz governorate and in Hajjah [13, 14]. Although the disease was recorded from different parts of north Yemen, many other south areas in Yemen Republic are not documented. Alswalaha area is one of the areas in Yemen, which is not documented. Therefore the current study aimed to determine the prevalence of CL in ten villages in this study area.

2. MATERIAL AND METHODS

2.1. Study area

Lahj province is located in the south-west of the Republic of Yemen. Al-Sawalha area belongs to Al-Maqatra district, it is located in the northern part of the Lahj province at longitude 44^0 55' and latitude $13^0$12' W, and rising from the sea in 2855 m. The area includes 16 villages. It is a mountain area (Fig. 1) characterized by the presence of many caves filled with water for long periods. They have mild climate and summer rainfalls. Most of the population lives in houses where the lower part of the house is used for cattle, which provides a favorable environment for the spread of insects, including insect vectors of *Leishmania*.

2.2. Methods

2.2.1. Sample collection

This study was carried out during the period from July 2011 - March 2012 and relied on clinical and laboratory diagnosis of 82 persons with lesions in different parts of their body. The samples examined in the medical laboratory in Turr Albahah hospital.

Figure 1. Map of Lahj governorate showing Al-Sawalha area (the red spot).

2.2.2. Microscopical analysis of skin scrapings

Small quantities of tissue obtained by skin scrapings were smeared on glass slides, air dried and fixed with methanol for a few seconds. The smears were stained with Giemsa stain. After 20 minutes of staining the slides were washed with tap water and air dried. The stained smears were examined under the microscope with a 40 x lens and with a 100 x oil immersion lens. If at least one amastigote with a distinctive kinetoplast was found the smear was declared positive. When no amastigotes were seen after 15 minutes of inspection, the smear was declared negative. Many of the patient smears were double checked.

2.2.3. Sample cultivation

Patient positive samples were cultured in Novy-MacNeal-Nicolle (NNN) medium as described by Evans [1] to confirm *Leishmania* positive smear. Cultured NNN media were incubated at 24-26 ˚C for 6 days. Thereafter, smears stained with Giemsa were prepared for microscopic examination.

2.3. Statistical analysis

Chi-square test and percentage of infections were applied to find the significant difference

between the data. *P*-value < 0.05 was considered significant.

3. RESULTS

Clinical diagnosis of suspected CL patients was parasitologically confirmed by demonstrating the presence of *Leishmania* amastigotes in skin lesions and by culture in NNN medium. Microscopic investigation revealed that 75/82 (91%) cases were positive for leishmaniasis. Prepared smears showed a great number of intracellular and extracellular amastigote stages. All the 75 positive cases were confirmed positive by culturing in NNN medium, and the smear from culture declares a promastigote stage. The infection was detected in both sexes, while males are often infected than females with infection rate of 60% and 40%, respectively (Fig. 2).

Lesions were recorded in different age groups, with the largest number of patients (38 cases) in the age group 10-20 years (Table 1).

A dry skin type of leishmaniasis due to *L. tropica* was more prevalent in the study area (65%) than the wet type (35%), as shown in Fig. 3.

The lesions were recorded from different parts of the body. The most ulcers were recorded on the face of 40 patients (45%), in the upper extremity of 25 cases (29%), and in the lower extremity of 17 cases (20%). Size of the lesions ranging between 3 mm to 5 cm (Figs. 4, 5 a-d).

The number of lesion per patient range from 1 to 3 and more. Most of the patients (55 persons, 73.3%) have single lesion, 15 (20%) patients have two lesions, while the remaining 5 (6.7%) patients developed 3 and more lesions (Fig. 6).

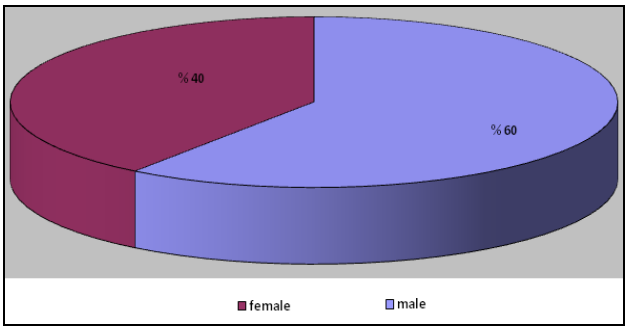

Figure 2. The distribution of CL among males and females in Allswalha area, Lahj, Yemen.

Table 1. The prevalence of CL among different age groups in Allswalha area, Lahj, Yemen.

Age group (years)	Total examined	Total infected	Positive rate (%)
<10	15	14	93.3
10-20	42	38	90.4
21-30	16	15	93.7
31-40	5	5	100
>40	4	3	75
Total	82	75	91.4

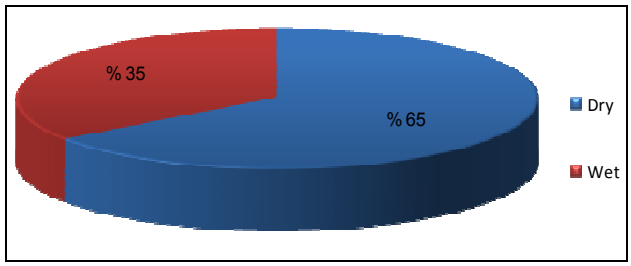

Figure 3. Types of CL lesions in infected patients in Allswalha area, Lahj, Yemen.

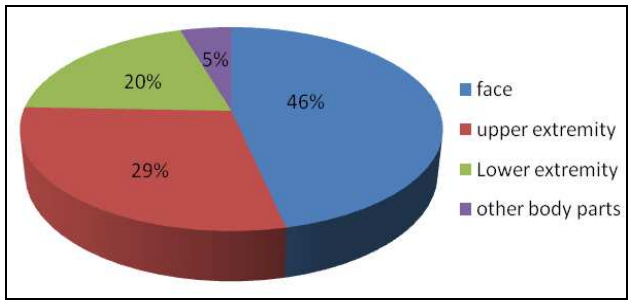

Figure 4. Percentage of CL lesions on the body of infected patients in Allswalha area, Lahj, Yemen.

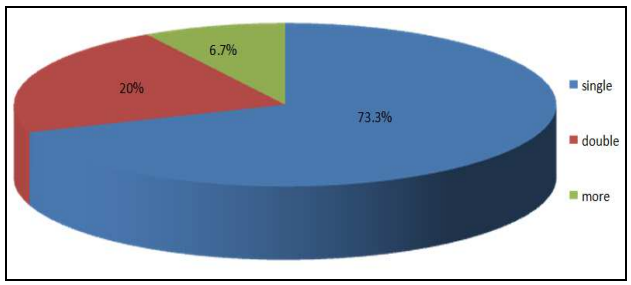

Figure 6. The rate of *Leishmania* lesions on the body of infected patients in Allswalha area, Lahj, Yemen.

The duration of the infection varied from one month to more than a year as described in Table 2.

Figure 5. (A) Women of 68 years old with dry CL on the nose. (B) Girl of 11 years old infected with double dry CL on the lips and cheeks. (C & D) Boy of 19 years old with multiple CL lesions on upper and lower extremity.

Table 2. Duration of CL among infected patients in Allswalha area, Lahj, Yemen.

Duration of infection (months)	No of cases	Rate of infection
1-6	54	72
7-12	18	24
>12	3	4
Total	75	100

4. DISCUSSION

Leishmaniasis is one of the most communicable diseases, which is clinically recognized from different areas in north Yemen. *L. tropica* and *L. infantum* were found in Sa'dah and Al Hudaydah governorates. They are responsible for most CL cases (85.80%) in north-western Yemen [14]. Present study show that CL is one of the chronic diseases in studied area of southern parts of Yemen. A majority of the patients showed „dry" type single noduloulcerative lesions on the face. Similar observations have been reported by others [15-19]. They reported that CL is usually noted on exposed parts of the body mainly face, arms and legs. On the other hand another study mentioned that the upper extremity was more infected [20].

The lesions were observed in different sites of the body, including unexposed sites, suggesting the possibility of multiple bites of vectors. Similar observations have been recorded by others [21, 22]. Our study recorded that males were more infected than females but it is not statistically significant ($p<0.05$). Sex difference can be attributed to the engagement of Yemeni males in outdoor activities and night duties that exposes them to sand fly bites more than females which is also noticed by others [23-25]. In addition, the dressing pattern among Yemeni women may play role in less exposing females to infection. We also noticed that females with lesions in their face refused to show their infection. They hesitated to be examined due to the

psychological aspects. This may lead to un proper comprisable data between males and females. Previously, Davies et al. [26] mentioned that the scars in the infected patients have deep psychological effects and could decrease the patients opportunities.

The study concludes that Al-Swalaha is one of the endemic areas in Yemen that is in need of high attention of the authorities and society and there is a need for a multicenter study to evaluate the extent of the disease and to determine different *Leishmania* species. In addition to those studies, focus on reservoir, vectors and therapeutic efficacy are needed.

ACKNOWLEDGEMENT

We are highly grateful to the laboratory workers in Tur Albahah hospital for their contribution in test performance.

AUTHORS' CONTRIBUTION

Both authors have equally contribution to studies and preparing of the manuscript. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

Authors declare that there is no conflict of interest.

REFERENCES

1. Evans TG. Leishmaniasis. Infect Dis Clin North Am 1993; 7: 527-546.
2. Mandell GL, Bennett JE, Dolin R eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 6th edn. Elsevier, 2005: 2428-2442.
3. Ashford RW. The leishmaniases as emerging and reemerging zoonoses. Int J Parasitol. 2000; 12-13: 1269-1281.
4. Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immun Microbiol Infect Dis. 2004; 27: 305-318.
5. Roberts MTM. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. Br Med Bull. 2006; 75-76: 115-130.
6. World Health Organization. Control of leishmaniasis. Sixtieth World Health Assembly WHA 60.13 agenda item 12.3., 2007. www.who.int/gb/ebwha/WHA60/A60_R13-en
7. Davies, CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. BMJ. 2003; 326: 377-382.
8. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007; 7: 581-596.
9. Al-Zahrani MA, Peters W, Evans DA, Chin C, Smith V, Lane RP. *Phlebotomus sergenti*, a vector of *Leishmania tropica* in Saudi Arabia. Trans Soc Trop Med Hyg. 1988; 82: 416.
10. Al-Jawabreh A, Schnur LF, Nasereddin A, Schwenkenbecher JM, Abdeen Z, Barghuthy F, et al. The recent emergence of *Leishmania tropica* in Jericho (A'riha) and its environs, a classical focus of *L. major*. Trop Med Int Health. 2004; 9(7): 812-812.
11. Saliba EK, Pratlong F, Dedet JP, Saleh N, Khoury SA, Oumeish OY, et al. Identification of *Leishmania* strains from Jordan. Ann Trop Med Parasitol. 2004; 98(7): 677-683.
12. Shani-Adir A, Kamil S, Rozenman D, Schwartz E, Ramon M, Zalman L, et al. *Leishmania tropica* in northern Israel: a clinical overview of an emerging focus. J Am Acad Dermatol. 2005; 53(5): 810-815.
13. Rioux JA, Daoud W, Pratlong F, El Kubati Y, Moreno G, Rageh HA, et al. Les complexes *Leishmania donovani* s.st. *Leishmania tropica* et *Leishmania major* en Republique Arabe du Yemen. In: Rious JA, ed. Leishmania: taxonomie-phylogenese. Applications eco-epidemiologiques. Montpellier: Institut Mediterran en d'Etudes Epidemiologiques et Ecologiques. 1986: 357-363.
14. Khatri ML, Haider N, Di Muccio T, Gramiccia M. Cutaneous leishmaniasis in Yemen: clinico-epidemiologic features and a preliminary report on species identification. Int J Dermatol. 2006; 46: 40-45.
15. Malik GM, Ahmed SM. Cutaneous leishmaniasis in Asir region, southern Saudi Arabia. Ann Saudi Med. 1991; 11: 591.
16. Morsy TA, Khalil NM, Salama MM, Hamdi KN, Al-Shamrany YA, Abdulla KF. Mucosal leishmaniasis caused by *Leishmania tropica* in Saudi Arabia. J Egypt Soc Parasitol. 1995; 25: 73-79.
17. Parks K. Epidemiology of communicable disease. In: Text book of preventive and social medicine. Jabalpur India, 2004; 24: 325-335.

18. Rahman FS, Abdullah H, Khan JA. The frequency of old world cutaneous leishmaniasis in skin ulcer in Peshawar. J Ayub Med College Abbottabad. 2009; 21(3): 72-75.
19. Ali A. Development of affordable molecular techniques for the diagnosis of leishmaniasis in Yemen. MS.c theisis, Liebg - University Giessen, 2009: pp. 77.
20. Jaffar H. Rifmpicin in cutaneous leishmaniasis - a therapeutic trial in Saudi Arabia. J Pak Assoc Dermatol. 2006; 16: 4-9.
21. Kubba R, El-Hassan AM, Al-Gindan Y, Omer AH, Kutty MK, Saeed MB. Dissemination in cutaneous leishmaniasis. I. Subcutaneous nodules. Int Dermatol. 1987; 26: 300-304.
22. Khatri ML, Shafi M, Benghazil M. Cutaneous leishmaniasis with unusual presentation. Ind J Dermatol Venereol Leprol. 1999; 65: 140-142.
23. Hamid LS. Cutaneous leishmaniasis. PhD Thesis. The University of Medicine and Pharmacy (Carol Davila) Bucharest. 1999; pp. 138.
24. Al-Mayah JY, Tarish HR, Janabi HS. Therapeutic effect of griseofulvin on cutaneous leishmaniasis. Kyfa Med J. 2000; 3(1): 62-601.
25. Uzun S, Uslular C, Yucel A, Acar MA, Ozpoyraz M, Memisoglu HR. Cutaneous leishmaniasis evaluation of 3074 cases in Cukurova region of Turkey. Brit J Dermatol. 1999; 140: 347-350.
26. Davies CR, Reithinger R, Campbell-Lendrum D, Feliciangeli D, Borges R, Rodriguez N. The epidemiology and control of leishmaniasis in Andean countries. Cad Saude Publica. 2000; 16(4): 925-950.