

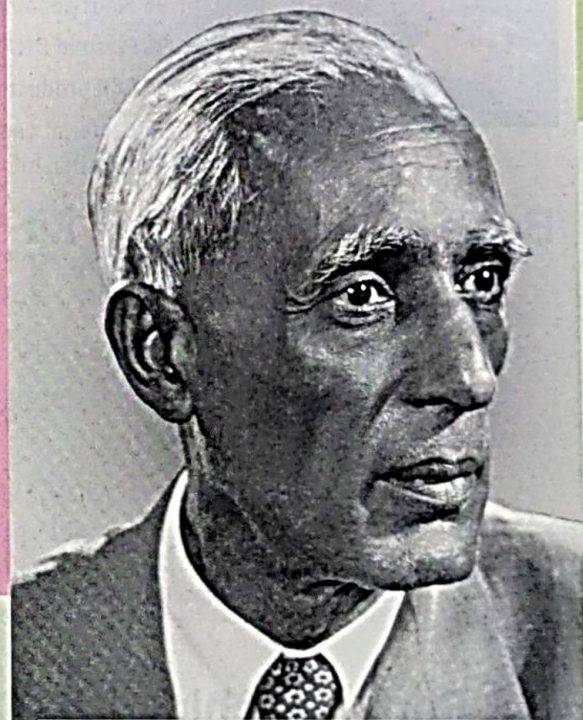
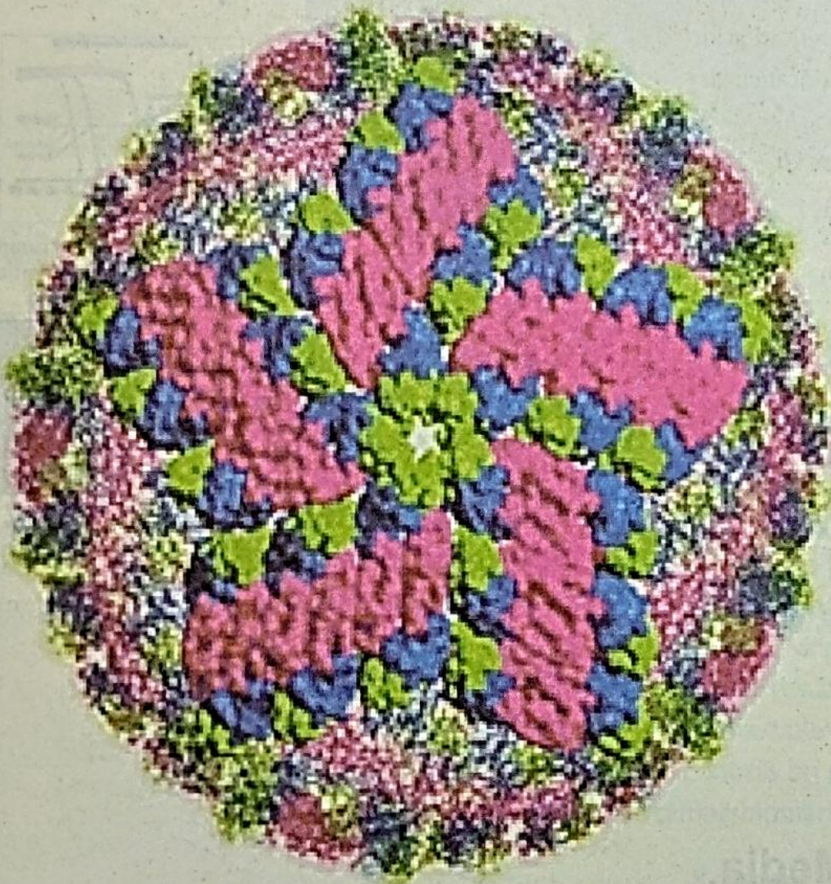


BULLETIN

OF

Haffkine Institute

D e n g u e



Major General
Sir Sahib Singh Sokhey

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SCIENTIST**

BULLETIN OF HAFFKINE INSTITUTE

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I, Dr. Abhay Chowdhary, hereby declare that the particulars given above are true to the best of my knowledge and belief.

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(-Sd)
Dr. Abhay Chowdhary
Editor-in-Chief
Bulletin of Haffkine Institute &
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“Look at the soil, just not at the seed”

Borrowing a line from *Padmabhushan* Dr. R. D. Lele, it gives me immense pleasure to bring forth this volume of the Haffkine Bulletin. Translated clinically, it means that one should look just not at the etiological agent, but also at the host response.

India being a tropical country is blessed with a plethora of flora and fauna. On the flip side, it is also a home to numerous pathogens and related diseases. We are witnessing an upsurge in the belief in alternative therapies and treatment, including Ayurvedic medicines. *Ayurveda* speaks of utilization of plants and other herbal formulations as medicines or therapy. It is critical to identify, isolate and correlate a medicinal activity hitherto attributed to a plant or plant product, but not demonstrated scientifically. Eliminating a pathogen through numerous mechanisms and modulating the immune response in a host are the two arms of this evaluation. This issue of the Bulletin deals with the first arm.

With this issue, we have tried to bring forth an association of many plants of medicinal value, with their activities against a variety of microorganisms. Something as common as a pomegranate having an anti-malarial activity,

or castor oil seeds showing potent activity against mosquito larva, implies a much needed drive towards identification of new actives. *In vitro* cultivation of Antamul, and proving the anti-oxidant activity of the callus is a tiny but important step in microcultivation of medicinal plants.

We have attempted to relive a small part of the glory of Major General Sir Sahib Singh Sokhey, by sharing his excellence with the young researchers who would be reading this issue. The Institute plans to commemorate the 125th birth anniversary of its first Indian visionary.

We hope this issue ignites the fire in many like-minded researchers, who would observe the environment around them more keenly, and would come out with novel research protocols and newer phytological entities for drug development.

Cheers and happy reading!



Dr. Abhay Chowdhary
Editor-in-Chief

Claiming the Mediciam

Arvind Patel*

Mr. X has a mediclaim policy. He has been paying his Mediciam Insurance premium regularly for the last 5 years. Suddenly his wife became sick and was hospitalized. He was aware about her health insurance policy. After his wife was discharged from the hospital and after all his personal and social problems got settled, he submitted his wife's mediclaim documents to the insurance company, expecting a reimbursement cheque within 15 days. But to his surprise and shock, the claim got rejected. After much running about, he got the claim settled. He got his reimbursement cheque almost after six months. This is not an isolated case. This happens with most of the health insured.

Being in-charge of Mediciam Department, I am witness to all such grievances. This has motivated me to write this article. Here I have made an attempt to explain the basic concept of mediclaim in easy to understand terminology.

I. HEALTH INSURANCE HAS THREE COMPONENTS

1. Health Insurance Companies:
 - a. Government insurance companies
e.g. New India Assurance, National Insurance, Oriental Insurance, United India Insurance, etc.
 - b. Private Insurance companies e.g. ICICI Lombard, Bajaj Alliance, HDFC, Aviva, etc.
2. TPA Companies i.e. Third Party Arbitrator e.g. MDIndia, TTK, Med Save, Paramount, etc.
There are more than 25 such companies.
3. The Insured: Individual, Family, Group or Corporate.

II. THE MOVEMENT OF THE MEDICLAIM FILE

The mediclaim file is first submitted to the respective insurance company by the health insured claimant by himself or through his agent. The file is then forwarded to TPA company, which will assess the validity of the claim based on the documents submitted, validate the policy, validate the treatment undergone and on satisfaction, settle the claim within the claim settlement parameter. The reimbursement cheque is then sent to the insured. The file then finally goes back to the respective insurance company.

III. TPA – THIRD PARTY ARBITRATOR

Third Party Arbitrator or TPA is the company appointed by the Insurance Companies to process and settle the insurance claim. Insurance companies pay service charges to the TPA. The TPA companies do not have their own rules set. They simply follow the policy terms and condition of the insurance companies, they interpret the policy terms and conditions, verify the Mediciam files and take decision about the claim status. Once the claim becomes payable, the TPA informs the respective insurance companies. Finally, with the permission of the insurance company, the TPA disburses the cheque to the Insured or the claimant. The cheque is normally posted to the claimant within 15 days.

The Following Steps must be adopted while claiming Mediciam:

1. When the patient gets admitted in the hospital, inform TPA and or Insurance company by email / Fax / SMS / Telephone within 24 hours.
2. On discharged from the hospital collect all the medical papers, bills, prescriptions for Medicines, X-ray Films with reports, MRI films with reports, 2D Echo Films / CD with reports, all pathological reports, all histopathological reports and certified indoor case papers. All the above reports should be in original.
3. If the patient expires, then along with the above documents also collect death summary.
4. If the patient is transferred to the other hospital, then the transfer note must be obtained from the hospital.
5. In Accident / Medico Legal Cases: All police complaint papers must be obtained and submitted with the Mediciam file.

While Submitting the Mediciam File:

Please submit the document in the following order:

1. Covering Letter – Original.
Mention the sickness/accident/reason for hospitalization in brief and a list of documents submitted. Show all the radiological films, CDs etc. and take them back after endorsement from the concerned authority of TPA.

2. Claim intimation proof.
3. Completely filled signed claim form with bank account details of proposer – Original.
4. Current year and first year policy copy – Photocopies.
5. Completely filled discharge card with date and time of admission and discharge – Original.
6. Certified Indoor case papers.
7. All Bills / Invoices / Receipts / Stickers of Implants – Original.

Please note: The mediclaim file containing all the above relevant numbered papers with tags must be submitted within 7 days after discharge from hospital to the Insurance Company / TPA as per guidelines with acknowledgement.

Always retain complete Photocopied set for your personnel record.

To conclude, if the health insured follows the above suggestions, I am sure claiming mediclaim will be very simple and without any hassle. If you have any suggestions, queries do not hesitate to contact me.

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Current Scenario of Medical Education in India– Suggestions for Change

G R Ekbote¹*

We are going to discuss the “Current Scenario of Medical Education in India and to make suggestions for change”, if any. National Knowledge Commission which was established by Hon’ble Prime Minister of India to recommend and undertake reforms in order to make India a knowledge based economy and society has made recommendations on Medical Education in India.

In its recommendations on Medical Education the National Knowledge Commission states as follows:

“Medical Education is a part of the whole system of education which is in crisis mode today and we are paying a very heavy price for inadequately investing in balanced growth for the social sector i.e. Health and Education. Health Education across the board in the country is directionless, unregulated, non-standardized. The quality, quantity and distribution of health oriented human resources being produced leaves much to be desired. With respect to quality, there is no effective monitoring or implementation of laid down standards, by any regulatory authority or well-designed need oriented educational programmes. The curricula have not been updated for years and there has been no attempt to emphasize the infusion of the curricula with cutting edge science technology of unprecedented knowledge explosion, to excite the young mind.

With respect to quantity and skill, it is not based on any disease surveillance data or any objective study of human resource requirements for making available trained health professionals as teachers, researchers, and for serving the health care needs of community by competent physicians, nurses or paramedical professionals.”

To become and remain globally competent, we have to review educational programmes and current scenario at undergraduate and post graduate level of medical education.

This can be considered under the following headings:

1. Regulatory Authority
2. Medical Institutes/Colleges
3. Curricula
4. Faculty
5. Students

In 1905, the well-known clinician and teacher William Osler suggested “To cover the vast information in medicine in 4 years is an impossible task. We can only instill principles, put the students on the right path, give methods, teach how to study and to discern early between essentials and non-essentials”.

Today with knowledge explosion in diverse fields related to medicine also vying for attention, these words resonate louder, in reforms we are ultimately interested in the impact of change on the clinical competence and patient outcome that today’s education can achieve.

Medical Education is important because it is responsible for the public health system and patient health care scenario in the vast country like India. Reforms in Medical Education also will help to update and improve the quality of training being imparted in our medical colleges to make current medical education nationally sensitive and globally competitive.

Following statistical data is important when we consider “Current scenario of Medical Education in India.”

It is interesting to note that the increase in number of private medical colleges has been responsible for this increase in number of medical colleges. In 1995, there were 47 private medical colleges, whereas by 2006, there are 131 private medical colleges. In the same period number of Govt. run medical colleges has increased from 109 to 131. Private medical colleges have got an urban bias.

¹Head of Unit General Surgery, B.J. Medical College, Sasoon Hospital, Pune, India; *Corresponding author

Year	1951	1991	2006	2011
Population of India	35 crores	80 crores	105 crores.	120 crores.
No. of Medical Colleges	30	146	262 Govt. – 131 Private – 131	300 (229 – Recognized Medical Colleges & 71 – Colleges permitted u/s 10A of Indian Medical Council Act, 1956)
Output of Medical students per year	Approx. 2000	Approx. 16000	Approx. 26000	Approx. 33528
No. of recognized doctors in India	61,800	-	6,46,000	7,97,764
Expenditure on health including medical education.	65 crores	19,818 crores	31,020 crores.	45000 crores
Doctor : Population ratio	17 / one lac	47 / one lac	59 / one lac	67 / one lac
No. of Primary Health Centers	4800	20,000	23,000	28,000 approx.
Govt. Hospitals	2,700	6,900	15,400	20,000 approx.

Large number of medical colleges are concentrated in 6 states only (viz.) Maharashtra, Andhra Pradesh, Karnataka, Tamilnadu, Kerala and Gujarat. These states cover 65% of total number of medical colleges & 70% of undergraduate medical seats. Therefore, it can be concluded that there is an uneven regional distribution of Colleges imparting Medical Education in various parts of country.

National Health Policy 2002 has mentioned about,

1. Uneven spread of medical education across various parts of the country.
2. Apart from uneven geographical distribution of medical institutions quality of medical education is highly uneven and in several institutions it is not upto the mark.
3. Medical education in the country does not address to the primary health concerns of the community, region and nation, which they have mandate to serve.

In 1978 at Alma Ata – International conference, World Health Organization (WHO) declared “Health for all through Primary Health Care as a major social goal”. The Alma Ata conference called on all states helping national policies, strategies and plan of action to launch and sustain “primary health care” as national health system. To achieve this goal medical education in our country will have to address to the needs of the society. It has to be vibrant. Medical Education in our country should be need based.

An interesting questionnaire was prepared and was submitted to the top academicians in various fields of Medical Education.

Following statements were read and academicians were asked to opine:

1. On the whole, medical education is very good and no significant changes are necessary.
2. On the whole, medical education is sound and requires only minor changes.
3. Medical education needs fundamental reforms although it has some good attributes.
4. There is so much wrong with medical education today that thorough reform is needed.

The result was very interesting.

1. 68% opined that fundamental changes are required in the curriculum of Medical Education.
2. 27% opined that only minor changes are required.
3. 5% opined that radical reform in Medical Education are required.

Therefore, majority of Medical Educationists opined that the fundamental changes are necessary. When 70% of top-most Medical academicians say that fundamental changes are required, it means that there are some weaknesses in the present system of Medical Education in India.

In brief, we will discuss about 5 important constituents of Medical Education.

1. Regulatory Authority:

An increasingly globalizing world demands a dynamic, responsive to change and well

informed Autonomous regulatory body to meet the emerging challenges for initiating, promoting, monitoring and enforcing standards of Medical education at all levels across the country. This body should be charged with the responsibility of directing, accreditation, standardizing, regulating and assessing medical education and the man power needs of all the strata of health care scheme. This regulatory authority / body should be set up by an Act of Parliament for co-ordinating and maintenance of standards in Health and medical education.

Medical Council of India is at present a only recommendatory body and it has got no genuine autonomy. It may be desirable that instead of creating new structure, to revamp, strengthen, enlarge the scope and empower with well-defined accountability in existing Medical Council of India to discharge above-mentioned responsibilities. This regulatory authority / body will be responsible to

1. To review of national health manpower requirements.
2. To ensure the creation of education institutional infrastructure and facilities or strengthening of such facilities.
3. To maintain standards in Health and Medical Education.
4. Regulation of Medical Education and accreditation of Medical Institutions / Colleges.
5. To consider establishment of one or more University of Health Sciences in the country.

National Knowledge Commission recommended that "if a regulatory authority is not put in place, medical education will be like a ship sailing in troubled water without navigational tools."

2. Medical Institutions / Colleges:

Many medical colleges not only lack infrastructure but also academic environment. Many medical colleges have not updated their infrastructure for number of years. These institutions / medical colleges must ensure that the environment is sufficiently rich that fosters broad academic purposes, a spirit of enquiry and moral values appropriate to a community of scholars. State Govts. should not disown their responsibility regarding medical education because it is a state subject. These medical colleges should not be starved for funds. It was proposed by National

Knowledge Commission that each state must identify at least one institution that can be seen as a center of excellence and role model for all the institutions in the State. The infrastructure, faculty, research laboratories, teachers training, library and state of art public care facilities must be provided in every medical college by the State Govt. Govt. medical colleges should have the infrastructure, research labs. Libraries as per the norms of the Regulatory Authority / Body. These institutions must be dynamic structures and initiate procedures for regularly reviewing and updating their structure and functioning and must rectify documentary deficiencies. These institutions must have a Dean, Academic head of the Institution who is qualified by education and experienced to provide leadership. This should have organizational structure which will reflect representation for academic, administrative staff, students and other stake holders. Medical colleges / Institutions must be regularly inspected and accredited from undergraduate and post graduate programmes.

The linkages with other medical colleges, ICMR, DST, DBT must be in place to sustain successful educational and environment.

3. Curricula:

The present curriculum which is followed in medical colleges needs to be revamped. The new one must ensure that the contributions of basic biomedical sciences that create an understanding of the scientific knowledge, concepts and methods fundamental to acquiring and applying these to clinical sciences be incorporated in an integrated manner.

Besides the strong foundations of basic and clinical sciences, the following essentials need to be incorporated or strengthened:

A) Skills :

1. Communication Skills.
2. Management skills.
3. Analytical Skills.
4. Research methodology.

B) Disciplines :

1. Social sciences
2. Medical Ethics and human management.
3. Health Economics.

C) New Courses :

1. Bio-informatics.

2. Genetics and genomics.
3. Molecular Biology.

Medical Colleges in India follow a rigid curriculum stuffed with a large burden of knowledge pertaining to the basic sciences and clinical Disciplines. Once qualified, students analyze and systemize this information and apply it to the care of patients. With expanding body of knowledge there is overburden of students with the “content information”. In order to grapple with these problems, it therefore essential to decide the “core content” which every student “must learn”, things that are “useful to learn” and “nice to learn” but don’t need same emphasis. A new strategy viz. “core with option” will have to be adopted. “Core curriculum” is to be developed by delineating basic knowledge, skills and attitudes which must be studied “before a newly qualified doctor” can assume the responsibility of a pre-registration house officer or a Medical Officer in Primary Health Center. “Options” provide areas to the students for study depending on individual needs and interest. Mastery of core ensures the maintenance of standard. The options provide in depth work and achievement of high level competency such as critical thinking. Another important fact of Medical Education which should be highlighted is increased importance which has to be placed on practical training and generic competencies. Concern has been expressed that undergraduate curriculum fails to fulfill the expectations despite students’ extensive exposure to clinical teaching. In addition to clinical competencies students must develop generic competency essential to their roles as Health Professionals, which will include communication skills, professional skills, abilities, organization skills, I T skills and doctor-patient relationship. One day, I was talking with a senior academician and he said regarding the curriculum of Medical Education in India that “We have tinkered long enough with the curriculum and content of the course but we have never considered suitability of a single uniform course when it is known that the final evolution as a General Practitioner or Specialist or a Medical Scientist requires different courses at the Under Graduate level itself.” It is time to consider the feasibility of multichannel courses (say a Hon. Courses like BA, B.Sc. in a subject eligible for specialization) to suit different groups. This would bridge the big gap between Under Graduate course and specialties.

Three such broad channels can be identified. :

1. A Family Doctor,
2. Specialist.
3. Medical Scientist.

In this way, it may be possible to plan manpower supply to fulfill the needs of a region or a State.

Many academicians or Medical educationists have proposed the idea of multi-disciplinary integration of Medical curriculum. This integration may be horizontal where topics taught separately in one level of course are taught together. (e.g. Anatomy, Physiology and Biochemistry of digestive system and be taught together for the 1st year students). This integration may be vertical. The topics can be taught by two or more departments together (e.g. Anatomy, Physiology and Surgery of Upper Gastro Intestinal tract can be taught by their departments together). Vertical integration is associated with earlier introduction of clinical work and incorporates basic sciences throughout the Under Graduate programme. This strategy is found to be more effective way of preparing students for their future roles. Far from being premature and interfering with the study of basic sciences, the evidence is that early clinical contact is handled well by students who see the relevance and value of what they are learning.

4. Faculty:

The selection of teachers must be strictly on merit. There should be Faculty development programmes and opportunity must be provided to enhance faculty members’ skill and leadership in education. The training should be mandatory and it is recommended that teachers’ training institutes should be established in the state and country as a whole. These institutes will help in up-gradation of teaching skills. At the entry level, every teacher should undergo a course in pedagogy.

Service conditions of the teachers should be strictly followed and incentive for promoting and research activities must be provided. Other incentives should include

1. fast track promotions,
2. some share of research grants obtained by faculty members.
3. Availability of sabbatical leave to update themselves at periodical intervals (after 5 years interval).

4. A security policy and tax incentives.

Faculty pay scales should be disconnected from the bureaucratic salary. Their cadres may be revised and fast track promotions for achievers must be available.

If those opting for a course in teaching, academics and patient care, are not encouraged to give their best, we are likely to lose them from our colleges/Institutes.

Teaching faculty should be permitted to invite guest faculty and should be able to attend national and international conferences regularly.

5. Students:

Admission and selection process should be transparent in public and private institutions. Although the admission process is transparent in Govt. Medical Colleges, it is not so in private medical colleges. The admission process in private medical colleges should be transparent and well regulated. It is suggested that there should be only one All India Common Entrance Test for all the students who will like to get admission in the medical colleges. This test can be conducted by CBSE. Fee structure in private medical colleges should be well regulated.

Admission process and fee structure in these colleges should be regulated by regulatory authority / body. This regulatory authority must have teeth and empowered by law to take action. Those medical colleges not having scientific and ethical infrastructure as per expectation do not deserve to exist. Poor quality commercial training shops producing poor quality manpower cannot be left loose on a helpless trusting public.

Student Assessment Policies:

At present, there is no standardization of student assessment and examination process across the country. There should be continuous assessment of the students throughout the course. (Internal Assessment)

Internship:

The Internship is a very important part of medical education but it has deteriorated over time and has reduced its relevance and value. The internship, currently unassessed, does not serve the purpose of giving hands on experience because the intern's focus and target is post graduate entrance examination and developing skill is far from their minds. It has become a

preparatory time for post graduate entrance examination with students staying at home and studying for post graduate entrance examination and not acquiring skills.

Therefore, it is suggested that the Internship year must be assessed and practice of students of studying in Internship year without attending clinical duties necessary to be changed immediately. The internship must be a period of acquiring and enhancing skill development and application of the knowledge acquired in community as well as hospital settings.

Establishment of New Courses:

It is suggested that new interdisciplinary courses should be established by the universities, e.g.

1. Medical and Engineering like Biophysics,
2. Medical and Sports Medicine,
3. Medical and Social Sciences,
4. Medical and Pharmacy ,
5. Medical and Hospital Management Courses. etc.

This phenomenon of establishment of interdisciplinary courses is currently occupying the minds of medical educationists and very soon it is going to emerge as a different branch. Even many top class academicians in the field of Modern Medicine and Indian Medicine are thinking of establishment of inter faculty courses e.g. Modern medicine and Indian Medicine.

Any successful development process must have a pyramidal structure with a strong horizontal base.

In terms of medical education there has to be strong base of basic scientists and clinical generalists who are backbone and stability of system. This is to be focused at higher levels by core specialists and lastly sub-specialists. At present there is no pyramidal structure. In fact the pyramid rests on a wobbly base of generalists and inversing trends for sub-specialists.

There is a need to introduce new courses in disciplines such as family medicine, geriatrics, specific branches of public health, emergency medicine, sports medicine, critical care, trauma care etc.

Autonomy to Medical Colleges:

The medical curriculum assessment system, teachers' recruitment policies, minimum

qualification for teacher etc. are currently regulated by MCI. However, some of the top medical colleges should be given sufficient autonomy in order to become world class institutions of higher learning.

I wish to state an example of 3 Engineering institutes in the State viz. (1) Govt. college of Engineering, Pune (2) VJTI, Mumbai. (3) Govt. Engineering College – Shri Guru Govindsinghji Institute of Technology and Research, Nanded were given academic autonomy.

Govt. College of Engineering, Pune and VJTI made rapid progresses in the development of infrastructure in their institutes after the autonomous status was granted by Govt. of Maharashtra. Govt. College of Engineering, Pune made collaboration with Japanese industry and it was successful in augmenting finances of the Engineering College by tune of approx. 30 crores. Prof. Dr. Mashelkar was the Chairman of Board of Directors of VJTI, Mumbai. He collected Rs. 8 crores from the alumni of VJTI and implemented several ideas to improve infrastructure facilities of VJTI. This was possible only after granting of autonomous status.

Can we think of any Govt. Medical College being granted Autonomy by the State Govt. in near future?

Maharashtra University of Health Sciences, Nashik was established in 1998. At present it has got approx. 250 Health sciences colleges affiliated with it. From our experience, sometimes it is difficult for

the University to administer, supervise, monitor health sciences education across the state. Secondly, stake holders (viz.) students, faculty and private organizations, find it difficult to reach the University headquarters in time. Therefore, it is suggested that another state university pertaining to the health science courses should be established in near future.

Change in medical education is currently a worldwide phenomenon. It is needed to prepare a doctor to fulfill expectations of society, to cope-up with exponential growth of medical and scientific knowledge, to inculcate physicians' ability for lifelong learning and to ensure mastery in Information Technology. Greater efforts will be required to be taken to orient teachers, trainers and planners to bring desired changes in Medical Education to produce need based human resources for health care system in the country. However such peadagogic shift from traditional to need based approach requires a fundamental change of mindsets, roles and commitments of educationists, planners and policy makers.

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Immune Response, Immunopathogenesis and Severity of DENV Infection

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INNATE IMMUNE RESPONSE

The innate immune response plays an important role in the recognition of pathogens, for example, in initiating a potent antiviral response to virus infection. Uptake of the pathogen by antigen-presenting cells (dendritic cells, macrophages) activates the pro-inflammatory response as well as the type-I IFN signaling pathway that is the upstream regulator of antiviral response genes. Type-I IFNs (α/β), which are produced by most cell types, play a central role in the innate antiviral response to a wide variety of viruses including DENV. However, since type-I IFNs can modulate maturation and activation of dendritic cells^[1, 2], they perform a vital function to bridge the innate and adaptive parts of the host immune response. Type-II IFN ($IFN\gamma$) also can induce expression of antiviral genes. However, most of the type-II IFN is produced by activated NK and T cells and not by virus infected cells.

NK cells are an important piece of the innate immune response partly by producing massive amounts of $IFN\gamma$. NK cells upregulate their expression of activation markers (CD69, HLA-DR and CD38), adhesion molecules (CD44, CD11a) and cytotoxic granules (TIA-1) early during the acute phase of dengue disease^[3]. However, the role of NK cells in the regulation of DENV infection has not been studied in depth. Higher levels of type-I and type-II IFNs have been detected in dengue patient serum during the acute stage of the disease^[4, 5]. Intravenous DENV (D2S10) infection in AG129 mice, which lack both type-I IFN and type-II IFN receptors, resulted in productive infection and $TNF\alpha$ -mediated lethal disease^[6]. Also, in the *in vitro* model of dendritic cell infection with DENV, $IFN\alpha$ and $IFN\gamma$ inhibited viral replication even though it has been shown that DENV infection inhibits type-I IFN signaling within infected cells^[7, 8, 9].

ADAPTIVE IMMUNE RESPONSE

The adaptive immune response involves antibodies and T lymphocytes. Depending on the serotype of DENV both aspects of adaptive immunity have been associated with secondary DENV infection-mediated pathogenesis^[10, 11]. Passive transfer of antibodies to E, NS1 and prM proteins has been shown to protect mice against lethal DENV challenge^[12, 13]. Cross-

reactive antibodies from previous DENV infections at sub-neutralizing concentrations can increase uptake of non-neutralized virus by binding to FcRII on cell surface^[14]. A study by Kliks et al. found high DEN-2 antibody-dependent enhancing activity as a risk factor for DHF^[15]. However, studies by Endy et al.^[16] and Laoprasopwattana K et al.^[14] did not find a correlation between titers of preexisting neutralizing DENV-antibodies and the severity of the secondary infections.

During secondary DENV infections, cross-reactive T cells presumably generated from the previous DENV infection proliferate more than the T cells specific to the newly infecting serotype^[17]. This phenomenon is called "Original Antigenic Sin". DENV serotype cross-reactive cytotoxic T cells show higher avidity for antigen and, as compared to DENV serotype-specific cytotoxic T cells, produce higher pro-inflammatory cytokines like $IFN\gamma$ and $TNF\alpha$ ^[18]. Furthermore, other cytokines like IL-2, IL-10 and IL-8, which can be produced by T cells, are elevated before the onset of plasma leakage^[5]. The altered immune response and higher production of pro-inflammatory cytokines during a heterologous secondary infection might play a role in vascular leakage and activation of the coagulation cascade.

PROTECTION

The longevity of the protection afforded by primary DENV infection against the homologous serotype is not completely understood. The major target for antibody response is to the E protein of DENV virion. *In vitro* antibodies against E protein both inhibit viral binding to cells and neutralize infectious virus^[19]. Furthermore, passive transfer of antibodies to E protein can protect mice against challenge with DENV^[20]. $IFN\alpha$ and $IFN\gamma$ were found to be critical for early and late immune response and resistance to DENV infection in mice, respectively^[21]. Further, a mouse-adapted strain of DENV injected peritoneally killed $IFN\alpha/\beta$ – and $IFN\gamma$ -deficient mice^[22]. $IFN\alpha$ can inhibit DENV replication in multiple cell types *in vitro*^[23, 24, 7]. However, $IFN\alpha$ cannot block DENV replication if added after DENV infection suggesting that DENV has evolved to counter $IFN\alpha$'s antiviral effects. $IFN\gamma$ also strongly inhibits DENV replication in

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DCs, but unlike IFN α , IFN γ treatment after DENV infection can still inhibit virus replication^[7]. Overall, immune responses and their effects to limit DENV replication are hypothesized to be protective against the development of the severe form of the disease.

CLINICAL STAGES OF DENV INFECTION

DENV infection, which has an incubation time of 3–7 days, is asymptomatic in a majority of individuals. Dengue fever (DF), Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are three major classifications for grades of severity of dengue disease. DHF is further sub-classified into grades I, II, III and IV by the World Health Organization (WHO). Grades III and IV constitute DSS. However, positive tourniquet test, one of the minimal criterion to diagnose a case as DHF has been unsuccessful in discriminating between DF and DHF effectively in multiple studies. In two studies, only 18% and 61% of the children with shock met the criteria classified by WHO for DHF^[25, 26]. Hence, prominent researchers in the field are in favor of modifying the current WHO standards of dengue classification^[27].

DENGUE FEVER

DF is a mild, self-limited febrile illness typically associated with the following symptoms: retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, leukopenia and headache. Most of the individuals recover after the acute febrile period, which may last from 2–10 days, without any specific treatment^[28]. There is lower risk of death in DENV patients presenting clinical symptoms for DF.

DENGUE HEMORRHAGIC FEVER

DHF is a more severe manifestation of DENV infection and can be caused by all four serotypes of DENV. A minority of people (~3%) infected with DENV develop this more severe form of disease according to the WHO classification criteria^[28]. During the febrile phase of the disease, patients present with similar clinical symptoms as DF. However, at the time of defervescence or shortly afterward, patients with DHF abruptly develop a vascular permeability/plasma leakage syndrome associated with thrombocytopenia, a bleeding diathesis. Vascular leakage and increased endothelial cell permeability resolve following the acute phase of the disease, suggesting dysfunction rather than structural damage to endothelial cells during DHF. A majority of cases of DHF are associated with secondary DENV infections^[29]

(DHF.CAB Int.Oxford,1997). Studies have found that children of ages 4 years and above are at higher risk of developing DHF following DENV infection while children under 3 years of age who were seropositive for DENV did not present clinical symptom of infection^[30]. However, the cause of this DENV infection-mediated pathogenesis is not well understood. “Original Antigenic Sin” and “Antibody Dependent Enhancement” models have been postulated to be the immunopathogenesis mechanisms that lead to increased severity of disease^[31, 32]. The cytokine profile resulting from heterologous immunity might add to the inflammation-induced immunopathogenesis^[31, 33]. Recent clinical studies have provided data suggesting that certain DENV genotypes are associated with DHF^[33]. The two models of pathogenesis are further explained in detail in the “Immunopathogenesis” section of the introduction.

DENGUE IMMUNOPATHOGENESIS

Activation of the immune response associated with the ADE and original antigenic sin models during heterologous DENV infection might be responsible for the immunopathogenesis observed during DHF/DSS. Antibodies produced during primary DENV infection bind to virions of heterologous DENV serotypes during subsequent (secondary) infections. *In vitro* studies have shown that macrophages but not DCs^[34, 35] efficiently uptake these virus-antibody complexes, which leads to an increase in viral replication by a phenomenon called antibody-dependent enhancement (ADE). Moreover, antibodies from a previous infection can also cause immune activation via innate immune reactions and complement activation^[36]. Levels of T cell expansion are also enhanced during secondary DENV infections^[31, 33]. However, expansion of lower-avidity memory T cells over high-avidity naïve T cells specific for the infecting serotype generates a more inflammatory and less cytotoxic T cell cytokine and chemokine response called original antigenic sin^[31]. Sub-optimal viral clearance and disturbance of homeostasis of the blood clotting system might occur. In addition, ADE might lead to increased DENV antigen presentation to T cells that might further contribute to the activation of inefficient T cells and the immunopathology of heterologous secondary DENV infections. Circulating levels of pro-inflammatory cytokines and chemokines like TNF α , IL6, IL-2, IL8 and MCP1 and regulators of inflammation like IL-10 and sST2 are further correlated with a severe outcome during acute stage of the disease^[37, 38, 39, 40, 41].

CONCLUSION

This overview on DENV and factors associated with the disease presented in the introduction shows that large bodies of experiments have been performed to understand the dengue disease. These studies have found that viral and many host factors correlate either with protection or pathogenesis during DENV infection. However, due to the absence of a reliable animal model, the significance of these correlations associated with the pathogenesis is not well understood. Hence, current focus in the field of dengue research is to understand the molecular changes effected by DENV infection to identify aspects of host immune response that can be induced or inhibited to develop a prophylactic or therapeutic vaccine against dengue.

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Necessity of Hib Vaccination in Pediatric Population: A Survey

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ABSTRACT

Introduction: Bacterial diseases continue to present a major threat to human health. There has been a remarkable progress in the prevention, control and even eradication of bacterial infectious diseases with improved hygiene and development of antimicrobials and vaccines. However, bacterial infectious diseases still remain a leading cause of global disease burden with high morbidity and mortality especially in the developing world.

Materials and Methods: Retrospective observational study was done using questionnaires involving 1,000 pediatricians all over India. Of the total, 100 pediatricians replied with filled questionnaires, nearly 40% of who have reported data from public general hospitals catering to poor patients.

Results: The findings from the questionnaire demonstrated that 35% pediatricians reported no growth in culture, while 41% had observed some bacterial growth. Isolation from CSF showed *N. meningitides* (35.9%) and *Haemophilus influenzae B* (Hib; 13.75%) as a causative organism of bacterial infections. Blood culture revealed *Haemophilus influenza B* in 9.66% samples and *S. aureus* in 28.51% samples. Sputum culture was difficult to grow although Hib was observed in 7% samples and *S. pneumoniae* in 32.2% samples. Growth of Hib ranged from 7.0–13.75% and 22.05–35.9% of *S. pneumoniae* in different body fluids.

Conclusion: This report suggests the need of vaccinating a child against Hib with specific comments on the urgency of its inclusion in the national schedule. It also amply suggests the necessity of including Hib vaccination in Indian Vaccine armamentarium considering the huge burden of pyogenic bacterial meningitis and pneumonia in pediatric population.

Keywords: Bacterial infections, *Haemophilus influenzae B* (Hib), pediatric population, vaccination

INTRODUCTION

In 1948, the US Secretary of State remarked “the conquest of all infectious diseases was imminent”. In 1962, F M Burnet, the Nobel Prize winner, made an optimistic statement “To write about infectious disease is almost to write of something that has passed into history..., the most likely forecast about the future of infectious disease, is that it will be very dull”^[1]. In December 1967, the US Surgeon General William Stewart declared victory by announcing “The war against infectious diseases has been won”^[1]. However, infectious diseases are a dominant public health problem even in the 21st century. The World Health Organization estimates 25% of the total 57 million annual deaths that occur worldwide are caused by microbes and this proportion is significantly higher in the developing world. In the landmark report to Institute of Medicine (1992) in the US, Joshua Lederberg and Robert Shope identified the emerging microbial threats and attributed to the emergence of new pathogens due to microbial evolution and adaptation, population growth, ageing population, poverty and malnutrition, internally displaced persons due to conflicts, environmental pollution and global warming^[1].

The global alliances for vaccines and immunization (GAVI) and many other health agencies and groups believe that *Haemophilus influenza B* (Hib) disease is a serious and significant public health problem in India. In a recent statement to the lay press, Dr

Panna Choudhury, President, Indian Academy of Pediatrics (IAP), commented, “*Routine use of Hib vaccine is an essential piece of a comprehensive pneumonia control strategy to reduce the disease's terrible burden on children.*” According to the data provided by UNICEF and WHO, Hib is a leading cause of acute bacterial meningitis and important cause of severe pneumonia in Indian children. Approximately 410,000 (19%) of under-5 deaths in India are due to pneumonia out of which an estimated 70,000 are caused due to Hib^[7]. Acute epiglottitis is also one of the life-threatening diseases because of its potential for sudden upper airway obstruction. It is a well-recognized entity in children but it is uncommon in adults and therefore is often misdiagnosed^[2]. Since the generalized use of Hib vaccine in children, presentations of acute epiglottitis disorder have decreased dramatically^[3]. It has also been reported that acute bacterial meningitis, para pneumonic empyema and otitis media are some of the commonest and predominant prevalent bacterial diseases seen in children^[4–6]. The present study estimates the existence of meningitis, otitis media, epiglottitis, pneumonia and empyema in children, their causative organism.

MATERIALS AND METHODS

Study population: A postal survey on pediatricians from India was undertaken in March 2010, with a view to evaluate the existence of bacterial infections in children. Out of a total approximately 1,000 pediatricians who received the questionnaire, 100

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Necessity of Hib Vaccination in Pediatric Population: A Survey

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ABSTRACT

Introduction: Bacterial diseases continue to present a major threat to human health. There has been a remarkable progress in the prevention, control and even eradication of bacterial infectious diseases with improved hygiene and development of antimicrobials and vaccines. However, bacterial infectious diseases still remain a leading cause of global disease burden with high morbidity and mortality especially in the developing world.

Materials and Methods: Retrospective observational study was done using questionnaires involving 1,000 pediatricians all over India. Of the total, 100 pediatricians replied with filled questionnaires, nearly 40% of who have reported data from public general hospitals catering to poor patients.

Results: The findings from the questionnaire demonstrated that 35% pediatricians reported no growth in culture, while 41% had observed some bacterial growth. Isolation from CSF showed *N. meningitides* (35.9%) and *Haemophilus influenzae B* (Hib; 13.75%) as a causative organism of bacterial infections. Blood culture revealed *Haemophilus influenza B* in 9.66% samples and *S. aureus* in 28.51% samples. Sputum culture was difficult to grow although Hib was observed in 7% samples and *S. pneumoniae* in 32.2% samples. Growth of Hib ranged from 7.0–13.75% and 22.05–35.9% of *S. pneumoniae* in different body fluids.

Conclusion: This report suggests the need of vaccinating a child against Hib with specific comments on the urgency of its inclusion in the national schedule. It also amply suggests the necessity of including Hib vaccination in Indian Vaccine armamentarium considering the huge burden of pyogenic bacterial meningitis and pneumonia in pediatric population.

Keywords: Bacterial infections, *Haemophilus influenzae B* (HiB), pediatric population, vaccination

INTRODUCTION

In 1948, the US Secretary of State remarked “the conquest of all infectious diseases was imminent”. In 1962, F M Burnet, the Nobel Prize winner, made an optimistic statement “To write about infectious disease is almost to write of something that has passed into history..., the most likely forecast about the future of infectious disease, is that it will be very dull”^[1]. In December 1967, the US Surgeon General William Stewart declared victory by announcing “The war against infectious diseases has been won”^[1]. However, infectious diseases are a dominant public health problem even in the 21st century. The World Health Organization estimates 25% of the total 57 million annual deaths that occur worldwide are caused by microbes and this proportion is significantly higher in the developing world. In the landmark report to Institute of Medicine (1992) in the US, Joshua Lederberg and Robert Shope identified the emerging microbial threats and attributed to the emergence of new pathogens due to microbial evolution and adaptation, population growth, ageing population, poverty and malnutrition, internally displaced persons due to conflicts, environmental pollution and global warming^[1].

The global alliances for vaccines and immunization (GAVI) and many other health agencies and groups believe that *Haemophilus influenza B* (Hib) disease is a serious and significant public health problem in India. In a recent statement to the lay press, Dr

Panna Choudhury, President, Indian Academy of Pediatrics (IAP), commented, “*Routine use of Hib vaccine is an essential piece of a comprehensive pneumonia control strategy to reduce the disease's terrible burden on children.*” According to the data provided by UNICEF and WHO, Hib is a leading cause of acute bacterial meningitis and important cause of severe pneumonia in Indian children. Approximately 410,000 (19%) of under-5 deaths in India are due to pneumonia out of which an estimated 70,000 are caused due to Hib^[7]. Acute epiglottitis is also one of the life-threatening diseases because of its potential for sudden upper airway obstruction. It is a well-recognized entity in children but it is uncommon in adults and therefore is often misdiagnosed^[2]. Since the generalized use of Hib vaccine in children, presentations of acute epiglottitis disorder have decreased dramatically^[3]. It has also been reported that acute bacterial meningitis, para pneumonic empyema and otitis media are some of the commonest and predominant prevalent bacterial diseases seen in children^[4-6]. The present study estimates the existence of meningitis, otitis media, epiglottitis, pneumonia and empyema in children, their causative organism.

MATERIALS AND METHODS

Study population: A postal survey on pediatricians from India was undertaken in March 2010, with a view to evaluate the existence of bacterial infections in children. Out of a total approximately 1,000 pediatricians who received the questionnaire, 100

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pediatricians replied, nearly 40% of who reported data from public general hospitals catering to poor patients. Pediatricians from all over India whose addresses were available were selected. Some addresses were incorrect and some had changed residences while few did not reply

RESULTS

A preliminary analysis revealed that the pediatricians had examined 1,420 cases of bacterial meningitis (other than tubercular) in one year, 5,046 cases of pneumonia and/or empyema, 5,624 cases otitis media and 392 cases of epiglottitis likely to be bacterial (Hib) in origin (Figure 1).

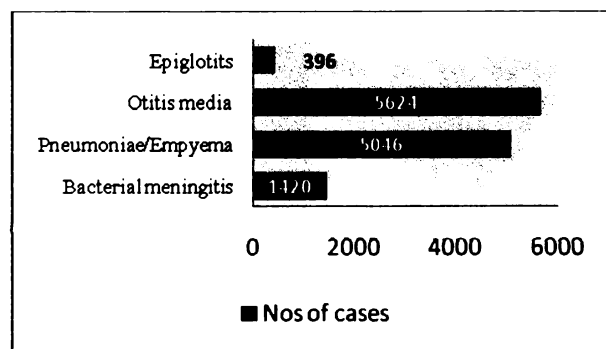


Figure 1: Bacterial infections in pediatric population

The questionnaire was also directed to identify the causative organism. 35% pediatricians reported no growth on culture, while 41% had observed some growth of the organisms. This is probably due to several difficulties involved in growing bacterial cultures. Despite that, Cerebrospinal Fluid (CSF) showed isolation of Hib in 13.75%, *S. pneumoniae* in 35.9%, *N. meningitidis* in 9.6%, and *Staphylococcus aureus* in 21.33%, *Streptococci* in 17.93% and *Pseudomonas* in 5.91%. Other organisms comprised of 19.2% cases (Fig. 2). Blood culture revealed Hib in 9.66%, *S. pneumoniae* in 22.05%, *N. meningitidis* in 6.5%, *S. aureus* in 28.52%, *Streptococci* in 16.85%, *Pseudomonas* in 12.70% and 30.85% other organisms (Fig. 3).

Sputum culture was difficult and Hib was grown in 7% cases, *S. pneumoniae* in 32.2% cases, *S. aureus* in 23.5% cases and *Pseudomonas* in 18.5% cases. Growth of organisms in Pleural fluid grew in similar numbers like sputum. Ear culture grew Hib in 12.5% of cases. Thus, there is a significant amount on invasive bacterial disease even in the absence of good culture facilities.

Growth of Hib and *S. pneumoniae* ranged from 7.0–13.75% and 22.05–35.9% respectively in different body fluids. However, Hib disease is

known to produce higher morbidity and requires longer duration of treatment, when compared to *S. pneumoniae*.

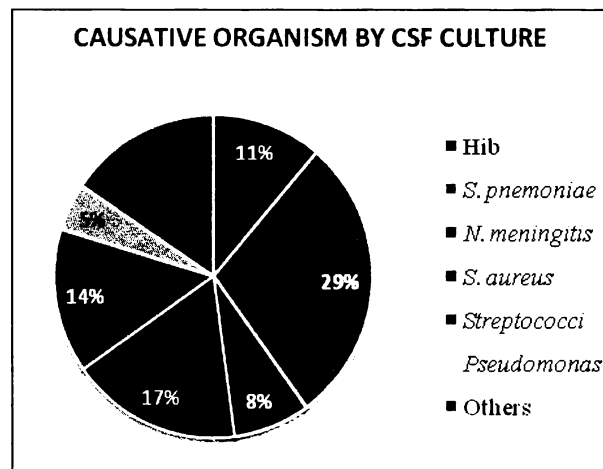


Figure 2: Isolation of Causative Organism of Bacterial Infections from CSF

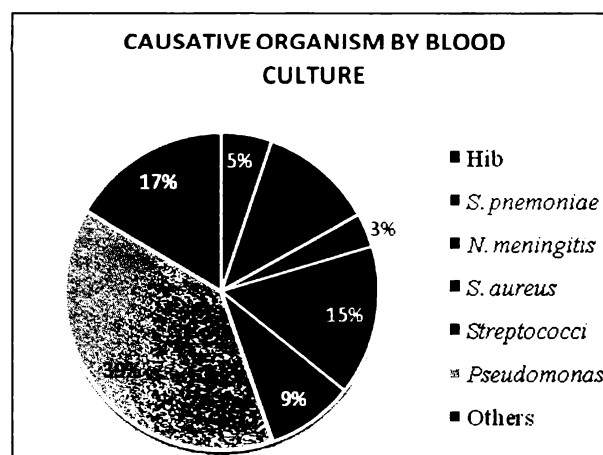


Figure 3: Isolation of Causative Organism of Bacterial Infection from Blood

DISCUSSION

Ninety-five percent of the pediatricians reported the need of vaccinating a child against Hib with specific comments on the urgency of its inclusion in the national schedule particularly because of the occurrence of meningitis, pneumonia and empyema in the lower socio-economic group, who need vaccination the most^[7-9].

This report amply justifies the necessity of including Hib vaccination in Indian vaccine armamentarium considering the huge burden of pyogenic bacterial meningitis and pneumonia in pediatric population.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Different Types of Reconstructive Surgeries for Basal Cell Carcinoma over Face

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ABSTRACT

Basal cell carcinoma (BCC) is the most common skin cancer all over the world due to exposure to sun light. About 30% caucasians develops BCC in their life time.

Due to its most common presenting site over the face above the line joining angle of mouth and ear lobule and due to extensive local spread it causes disfigurement of face. Therefore it is important while operating these patients to remove all cancer tissue and to do cosmetically acceptable reconstruction. For reconstruction of facial region different methods are used according to the region of face involved and size of the defect after wide local excision.

This study deals with classification of patients according to the region of face involved. This study also deals with different reconstructive options available for acceptable reconstruction. It also deals with prognosis & recurrence of BCC as recurrence is high in regions of nose and facial structures may be because of inadequate removal of tumour.

Keywords: Basal cell carcinoma, Reconstructive Surgery, Facial Region, Rodent Ulcer, Tear Cancer

INTRODUCTION

Basal cell carcinoma is the most common skin cancer. It is also known as Rodent ulcer or tear cancer. It rarely metastasizes or is fatal, but it is still considered malignant because it can cause significant destruction & disfigurement by invading surrounding tissues^[2]. Statistically approximately 3 out of 10 Caucasians develop a basal cell carcinoma in their lifetime^[3]. 80% of all the cases BCC are found on head, neck & face^[3]. It is due to direct exposure to sunlight^[3].

Classification^[4, 5]

For simplicity BCC can be divided in to 3 groups

- a) Superficial basal cell carcinoma
- b) Infiltrative basal cell carcinoma
- c) Nodular basal cell carcinoma.

Signs & Symptoms:

Patients present with shiny, pearly nodule. However superficial cancer may present as a red patch. Infiltrative cancer can present as skin thickening or scar tissue^[2].

Epidemiology:

It is the most common skin cancer. It is common in fair-skinned individuals with family history. There is increase in incidence close to equator & high altitude^[3].

Distribution:

In 2/3rd patients of BCC occur over sun exposed areas^[2, 3]. Most common site is over face.

Diagnosis Excision biopsy under local anaesthesia⁶.

Pathophysiology:

BCC develops in basal cell layer of skin. Sun light exposure leads to formation of thymine dimers, a form of DNA damage. While DNA repair removes most UV-induced damage, not all cross links are excised. There is therefore cumulative DNA damage leading to mutations. Sunlight also depresses local immune system. Mutation of PTCH1 gene & SMO gene also causes basal cell carcinoma^[7].

Treatment:

- Standard surgical excision with reconstruction
- Chemotherapy with 5% Imiquimod or 5-fluorouracil
- Immunotherapy
- Radiation therapy
- Photodynamic therapy
- Cryosurgery
- Electrodesiccation and curettage
- Prognosis is excellent if treatment is done early

MATERIALS AND METHODS

Patients were selected from OPDs of Surgery, Plastic surgery, Ophthalmology, ENT & Skin. Also from the indoor patients of all departments.

Inclusion criteria:

Patient should have lesions over head, neck or face.

Lesions should be surgically operable and patient should be fit for anaesthesia.

A total (15) no. of patient studied who were admitted to ENT and surgical wards. These patients having

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BCC of the facial region were admitted for excision of these lesions & reconstruction of the defect. BCC was diagnosed by the typical history of slow growing lesions of various macroscopic appearances, and it was later confirmed by histopathological examination. These patients were examined fully; information regarding type, number, the site, size & shape of the lesion were recorded. Preoperative photographs were taken too. The patients were grouped according to the site, size, and number of the lesions over the facial region.

Reconstruction Methods:

A total of (10) patient who were admitted to the Specialized Surgical wards in B. J. Medical College; Sasoon Hospital Pune, with BCC over facial region were treated by different reconstructive methods as following.

Methods of reconstruction:

Reconstruction of the defects was carried out for all patients using primary closure of defect, grafts or flaps under local or general anaesthesia. Skin graft were mainly used when the tumour was superficial & did not involve underlying bone or cartilage.

Grafts used in reconstruction were the split thickness skin grafts (STSGs), full thickness skin grafts (Wolfe graft).

Skin grafts were used in 3 patients. Flaps were carried out in 7 patients with larger defect or whenever the bones or cartilages were exposed. Excision of BCC with primary closure was done in 5 patients.

Different flaps used:

1. V-Y advancement flaps (2 patients)
2. Forehead flaps (2 patients)
3. Cheek advancement flap (1 patient)
4. Nasolabial flaps (2 patients)

For full thickness lesion, both flaps & grafts (mixed type) were used for reconstruction. Primary Sutures: were used in reconstruction of small lesions less than 1.5 cm in diameter (5 direct patients). In these patients skin incisions are taken along the Langer's line & skin is closed by subcuticular stitches.

Types of reconstruction for facial region defects after wide local excision:

The primary reconstructions were carried out for lesions with 2 cm. or less in diameter; the tumour was excised with 5 mm safe margin & reconstructed immediately after excision of the lesion. Secondary reconstructions were carried out for lesions more than 2 cm in diameter, the tumour was excised with 5mm safe margin & reconstructed later on, until the result of histopathology confirmed the

clearance of the defect from malignant cells (during this period the patient was daily dressed with povidone iodine 10%).

Follow up: All patients were followed up weekly for the first month, and then monthly for the first year, this included local examination, & photographs were carried out.

Selected patients were evaluated by:

1. History
2. General Examination
3. Excision or Preoperative skin biopsy
4. Lab investigations
5. Anaesthetic fitness for surgery
6. Wide local excision & postoperative histopathology reports
7. Aesthetic outcome
8. Type of reconstruction used
9. Recurrence



Figure 1

- 1 – Forehead unit (1a central subunit; 1b lateral subunit 1c eyebrow subunit)
- 2 – Nasal unit
- 3 – Eye lid units (3a lower lid unit; 3b upper lid unit; 3c lateral canthal subunit; 3d medial canthal subunit)
- 4 – Cheek unit (4a medial subunit; 4b Zygomatic subunit; 4c lateral subunit; 4d buccal subunit)
- 5 – Upper lip unit (5a philtrum subunit; 5b lateral subunit; 5c mucosal subunit)
- 6 – Lower lip unit (6a central subunit; 6b mucosal subunit)
- 7 – Mental unit
- 8 – Auricular unit
- 9 – Neck unit

OBSERVATIONS & DISCUSSION:

No.	Age/sex	Type of BCC on histopathology	Site	Size of lesion (cms)	Size of defect (cms)	Reconstruction used
1	45/m	Nodular	Cheek	1.3	1.8	Primary closure
2	58/m	infiltrative	Cheek extending up to lower eyelid	4	5	Forehead flap
3	48/f	infiltrative	Temporal	0.8	1.5	Primary closure
4	62/m	superficial	Lower eyelid	2.3	3	v-y cheek advancement
5	41/m	superficial	Cheek	3	4	split thickness skin grafting
6	47/f	superficial	Maxillary prominence	2	2.5	Full thickness skin grafting
7	52/f	Nodular	Zygomatic	1	1.5	Primary closure
8	46/m	infiltrative	Nose	2	3	Midline forehead flap
9	58/m	infiltrative	Lower eyelid	2.8	3.5	v-y advancement with ear cartilage
10	57/m	Nodular	Side of ala of nose	1.5	2.2	Cheek advancement
11	64/f	superficial	Cheek	0.7	1.4	Primary closure
12	61/m	superficial	Cheek	2.9	3.6	Split thickness skin grafting
13	54/m	Nodular	Forehead	1.1	1.6	Primary closure
14	53/f	Nodular	Nose	2.1	2.6	Nasolabial
15	56/m	infiltrative	Nose	1.7	2.4	Nasolabial

Table 1

DISCUSSION:

Full-thickness skin grafting or split-thickness skin grafting for cheek defects is a relatively simple, effective method of providing coverage. Full-thickness skin graft harvested from the postauricular, and supraclavicular areas produce best results in terms of color, character, and texture match. Skin originating in other areas may suffer from color mismatch, and mismatch in the skin thickness can cause lack of proper contour relative to neighboring tissue. Particular attention should be given when skin graft is considered for defect closure adjacent to eyelid, nasal margin, or oral commissure due to concern of skin graft contraction

The nose is a common location of BCC tumors. Numerous reconstruction methods have been devised and utilized according to the characteristics of the defect. Primary closure is the most straightforward method if the defect is small. Although skin grafting is a simple option, it is not a suitable reconstruction method for most defects of the nose, also when large surface to cover & when bone or cartilages are exposed because it is difficult to obtain a good texture and color match. A local flap is a more favorable reconstruction method for the lower portion of the nose, where the skin is thick and dense with sebaceous glands. However, if a defect of the nasal tip or the ala is superficial and too large to cover with a local flap, a full-thickness skin graft can be used, especially when the skin is relatively thin and sebaceous glands are sparse.

Local flaps which we used for reconstruction of the nose are listed as follows:

1. Nasolabial flaps

The superiorly based nasolabial flap is useful for defects of the nasal sidewall, ala, and tip, while the inferiorly based nasolabial flap is useful for defects of the upper and lower lip, nasal floor, and columella. The blood supply to this flap is excellent due to perforating branches of the facial artery. The color and texture are excellent matches, while the donor site scar is acceptable in the nasolabial sulcus. Using a template defect, a flap is designed on the nasolabial fold. It is best to make the flap exactly match the defect size. The medial incision for the flap follows the nasolabial sulcus, and the lateral incision is placed no higher than the level of the inferior defect margin. The flap is elevated in the subcutaneous plane, and the plane goes deeper as it proceeds superiorly. The flap is rotated counterclockwise on the right side and transferred to the defect.

2. Midline forehead flap

The midline forehead skin flap can serve as a cover for any nasal reconstruction from severe tip and ala loss to a total nasal defect. Using this flap, esthetic and functional reconstruction can be achieved by creating a nose that blends well

with the face. The seagull-shaped flap is based on one of the supratrochlear vessel bundles. Its vertical axis is placed over the midline of the forehead, and the wings are designed to lie in natural transverse creases. Local flaps are turned over and carried down to the septal support for lining. The forehead flap is elevated and transposed 180° to cover the new nose. The body of the seagull lies along the bridge, the wings curl at the ala and turn into the nostril sills, and the seagull head and neck creates the tip and columella. The forehead donor site is primarily closed.

3. V-Y flap

Subcutaneous V-Y flaps for the reconstruction of nasal defects have been gaining in popularity, especially in nasal dorsum reconstruction. The flaps have also been used in ala nasal reconstructions, for defects generally limited to less than 1.5 cm. in diameter and not *involving the rim*. The advantages of having similar tissue in the same operative field, with an excellent blood supply, make the V-Y flap a common choice for nasal reconstruction. The area of lesion excision and the flap is marked preoperatively. However, this flap has limitations, particularly in instances involving the inferior margins of the nose near the anterior nares. Some notching along the alar rim may occur and, in younger individuals, would probably be severe. For repair of the nostril rim, this flap may not be effective.

4. Cheek advancement flap

This flap use when defect is over lateral side of nose or below the lower eyelid. Flap is advanced below upwards in subcutaneous plane have advantage of using local tissue for cover so good colour match & also have good blood supply.

RESULTS

Follow up of operated patients done ranging from 6 weeks to 9 months 15 patients were analysed on the basis of functional outcome as eye opening, nasal opening or breathing without any discomfort. In 5 patients in whom primary closure was done scar was rarely visible & patients had great satisfaction. One patient in whom Cheek advancement flap was done for lesion around lower eyelid had complaint of ectropion rest of the patients had no complaints regarding opening & closure of eye. In patients with v-y plasty & nasolabial flaps there was good aesthetic outcome as patients were satisfied with good colour match. In cases of forehead flaps there are chances

of more improvement by division of pedicle & debulking of the flap. At the end of 9 months of follow none of the patient had recurrence.

CONCLUSIONS

In our study we found that most common type of bcc is nodular one and nose is the most common site. It occurs after 40 years of age. Local flaps used for reconstruction gave best results. Also it is easy and cosmetically acceptable. Recurrence rate might be lower if two different teams do excision and reconstruction.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Assessment of Hypolipidemic activity of *Anethum sowa* seeds against Triton X-100 induced Hyperlipidemia in Wistar Rats: A preliminary study

Pushkar Athalekar¹, Monu Ojha¹, Pallavi V. Ansulkar¹, Amruta Naik¹, Mrunal Ghag² and Abhay Chowdhary^{1,2*}

ABSTRACT

Introduction: *Anethum sowa* [Dill] has been used traditionally for various medicinal properties. The present study aims to study the protective or preventive Hypolipidemic activity of *Anethum sowa* seeds [methanolic extract] against Triton X-100 induced Hyperlipidemia..

Materials and Methods: The *Anethum sowa* seed powder was subject to successive Soxhlet extraction using three different solvents i.e. Hexane, Dichloromethane and Methanol. Further acute toxicity studies and Hypolipidemic activities of *Anethum sowa* seed extract was tested in male Wistar Rats against Triton X-100 induced Hyperlipidemia.

Results and Conclusions: *Anethum sowa* methanolic extract did not show significant Hypolipidemic activity against Triton X-100 induced Hyperlipidemia. This study was a preliminary analysis.

Keywords: *Anethum sowa*, Hypolipidemia, Hyperlipidemia, Cholesterol, Triton X-100, Wistar rats

INTRODUCTION

Atherosclerotic diseases are a leading cause of disability and death worldwide and two thirds of the cases are associated with Hyperlipidemia. Approximately 10 % of global population is affected by Hyperlipidemia^[1]. It results from abnormalities in lipid metabolism leading to increase in Serum LDL, Cholesterol & Triglycerides levels. Hyperlipidemia is a primary risk factor in the development of cardiovascular disease. With fast changing food habits in Indian population and there more and more inclination towards western food ,the rate of heart disorders in Indian population is increasing significantly. Therefore, the search for natural substances capable of lowering blood cholesterol is ongoing in the field of nutrition. Several drugs such as statins, fibrates, Bile Acid Sequestrants etc are used in Treatment of Hyperlipidemia^[2, 3]. It has been reported that drugs like statins and fibrates have several side effects and not recommended for long term use^[4-6].

Ayurveda, the ancient Indian system of health care and medicine, has a well-organized *materia medica* in which plants form a dominant part. A key illustration of the exploitation of this knowledge towards the development of better natural and effective Hypolipidemic agents accompanied with low side effects. Many natural herbs such as *Ocimum Sanctum*, *Pisum sativum*, *Carica papaya*, *Curcuma longa* have proven Hypolipidemic activity. Hypolipidemic activity in many polyherbal formulations is also reported^[7-10]. Indian Dill (*Anethum sowa* /*Anethum graveolens*) is an annual, globrous, aromatic herb consumed widely as green

leafy vegetable in India. It is rich in number of phytochemicals and Micronutrients and possesses several therapeutic properties^[11]. Literature reports that studies have been conducted on the aerial sections of the plant for its Hypolipidemic activity. Antihyperlipidemic activity has been reported in leaves of *Anethum graveolens*^[12].

In present study; we use the *Anethum sowa* seeds to investigate its Hypolipidemic activity against Triton X-100 induced hyperlipidemia. Literature reports that Triton X-100 is a inducer of choice for acute Hypolipidemic studies as it successfully induces Hyperlipidemia by raising the TC, TG, LDL-C levels in serum^[13]. Our study mainly aims to investigate the protective activity of *Anethum sowa* seeds crude methanolic extract i.e. whether the plant extract prevents Triton X-100 from inducing Hyperlipidemia. The assessment was a preliminary analysis.

MATERIALS AND METHODS

Plant Material:

Anethum sowa seeds were procured from an authentic registered Ayurvedic shop, was washed, shade dried and then powdered.

Crude Extract Preparation:

The *Anethum sowa* seed powder was subject to successive Soxhlet extraction using three different solvents i.e. Hexane, Dichloromethane, Methanol [from Non Polar to Polar]. Percentage yield of all three extracts were calculated and the extracts were stored 4°C until further use^[14].

In vivo Study:

Acute toxicity studies:

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The animal study was conducted after the permission granted by the Institutional Animal Ethical Committee. Study was performed as per OECD guidelines 420^[15]. Male Wistar rats (180–250 g) were kept under controlled environmental conditions of temperature ($22^{\circ} \pm 3^{\circ}\text{C}$) and humidity (30–70%) with 12 hours light/dark cycle and were given access to food and water *ad libitum*. Following administration of a single dose (2000 mg/Kg of *Anethum sowa* methanolic extract), the

animals were observed for clinical symptoms for 30 minutes, at hourly intervals for next 24 hrs and then continuously for 14 days for signs of convulsions, circling, tremors and mortality. Body weight was recorded on 0th Day and 14th Day.

Assessment of Hypolipidemic Activity:

Animals were acclimatized for 1 week prior to the main study.

Animal grouping was as follows (Table 1):

Group number	Group name (5 rats/group)	Dose	Treatment/s
1	Vehicle Control	Distilled water	Day 1 to Day 7
2	Positive Control	Atorvastatin [30 mg/kg]	Day 1 to Day 7 P.O
3	Hypolipidemic Control	Triton X-100 [100mg/kg]	Day 8
4	Test Group 1	Anethum sowa [250mg/kg]	Day 1 to Day 7 P.O.
5	Test Group 2	Anethum sowa [500mg/kg]	Day 1 to Day 7 P.O.

Table 1: Animal grouping

The study was conducted for 11 days. Blood was collected initially [Day 0], Day 8 [0 hrs before triton Induction] and successively on Day 9, 10, 11 i.e.24 hrs,48 hrs and 72 hrs after Triton injection respectively via retro-orbital route under light ether anesthesia. Serum Total Cholesterol [TC], Triglycerides [TG], High Density Lipoproteins [HDL] parameters were analyzed. S.G.O.T and S.G.P.T parameters were also studied since Triton X 100 may cause liver Toxicity. Based on the above parameters VLDL, LDL were calculated using Freidwalds formulas^[16] as follows:

$$\text{LDL-C} = \text{TC} - [\text{VLDL} - \text{HDL-C}]$$

Where VLDL = TG/5

The bodyweight and feed consumption of the animals were recorded from Day 1 to Day 11.

Statistical Analysis:

All the results were expressed as mean $\pm$ SD. Statistical Analysis was carried using One Way Analysis of Variance [ANOVA] and Boneferroni's Multiple Comparison test^[17].

RESULTS

In vivo study for Hypolipidemic activity of *Anethum sowa*:

Acute toxicity study: No mortality occurred in any of the rats at the dose of 2000 mg/kg indicating its safety. There were no changes in nature of stool, urine and eye color in any of the treated animals. The body weight of each rat was assessed on 7th Day & 14th Day and decrease in body weight was observed on both the days. There was a decrease in Feed Consumption & locomotor activity and increased lethargic behavior of all the animals.

Hypolipidemic activity

Serum Analysis Report: (Values expressed as Mean $\pm$ SD for all parameters)

Statistical significance observed between group 4 and group 5 at 5 % level of significance (Figures 1–5).

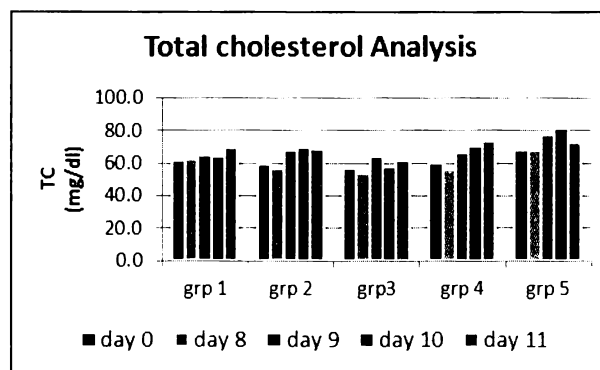


Figure 1: Total Cholesterol Analysis (mg/dL)

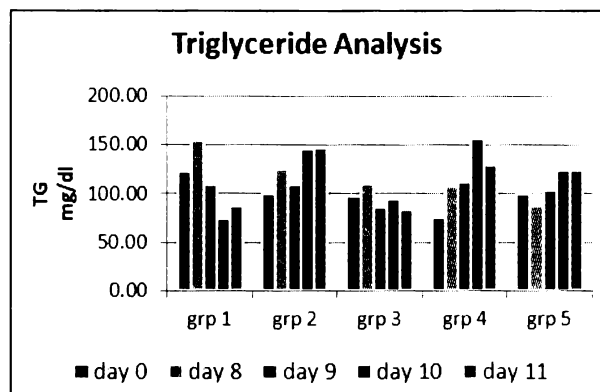


Figure 2: Triglycerides Analysis (mg/dL)

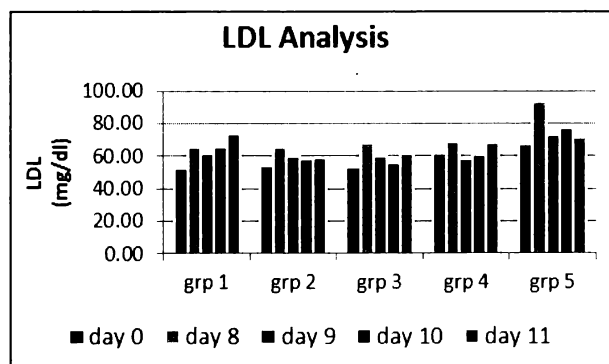


Figure 3: Low Density Lipoprotein Analysis (mg/dL)

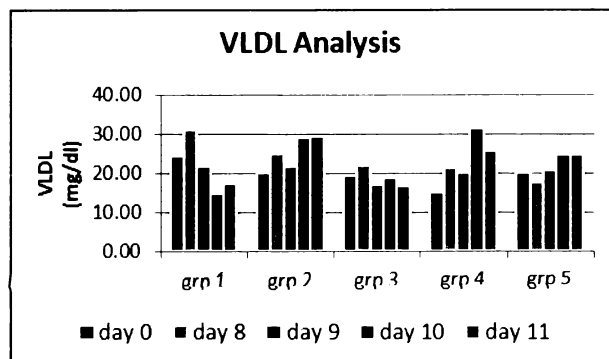


Figure 4: Very Low Density Lipoprotein Analysis (mg/dL)

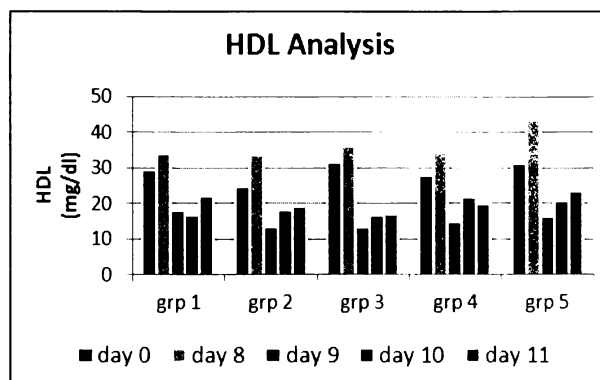


Figure 5: High Density Lipoprotein Analysis (mg/dL)

Groups	Day 3	Day 5	Day 7	Day 9	Day 10	Day 11
1	59.8	54.8	50	31.8	18	32.2
2	53.2	51	50	7	4.6	7.8
3	59.2	47.6	49.2	12.4	9	11.8
4	57	51.4	50.6	11.2	8.6	10.2
5	59.8	56	53.8	12	9.2	9

Table 2: Feed Consumption of Wistar Rats; Values expressed as Mean $\pm$ S.D

There was a drastic reduction in feed consumption of all the groups when compared to group 1 on Day 9, 10 and 11.

Groups	Day1	Day 3	Day 5	Day 7	Day 9	Day 10	Day 11
1	343.2	356.4	359.6	369.4	364.8	355.4	363.6
2	368.4	372.4	377.4	384.6	368.4	350.6	345.4
3	364.6	380	382.8	383.6	365.6	356.2	351.2
4	356.6	370.2	374.2	379	363.4	357.5	351.2
5	368	378.4	383.6	382.8	369.2	365.25	361.6

Table 3: Body Weight of wistar rats

There was no significant reduction in Body weight of all the groups when compared to group 1 on Day 9, 10 and 11.

DISCUSSION

It is known that elevated serum Lipid levels are associated with CHD and are primary risk factors for atherosclerosis. Several strategies are known to combat cardiovascular diseases such as dietary control, regular exercise and use of synthetic drugs like statins. But since these drugs have adverse side effects, the herbal library is being explored for search of newer molecules which will act as better Hypolipidemic drugs with minimal side effects. *Anethum sowa* is one of the Herbal plants with proven benefits to cardiovascular, reproductive, gastrointestinal system. Previous studies claim that *Anethum sowa* contains Phyto-chemicals like flavonoids due to which it acts as an Antioxidant and an Hypolipidemic agent.

The mode of action of Triton X-100 is probably due to inhibition of Lipoprotein Lipase activity. Inhibition of LPL causes increase in triglyceride and Cholesterol since LPL hydrolyses lipids in Lipoproteins. It is concluded that inhibition of LPL activity by Triton is due to modification of the substrate such that the enzyme can no longer act. Further, it is suggested that the chief cause of Triton Hyperlipidemia is coating of the plasma lipoproteins by Triton resulting in their faulty catabolism. Previous studies claim that Triton X-100 is an effective inducer of Hyperlipidemia. Triton X-100 has been used for acute Hypolipidemic studies since it increases TC, TG and LDL values efficiently.

In this study, Cholesterol, LDL and triglycerides values got rise after Triton X-100 Injection but

the results were not statistically significant at 95% confidence interval. Atorvastatin [standard drug] showed better protective activity as compared to the plant extract. Dose at 250 mg/Kg of plant extract showed better protective activity as compared to that at 500 mg/ Kg against cholesterol parameter. There was decrease in HDL values after 24 hrs of Triton Injection. Plant extract dose 500 mg/ Kg showed protective activity by raising the HDL values although it was not statistically significant at 90% confidence interval. There was an increase in S.G.O.T & S.G.P.T values after Triton Induction which may be an indication of liver toxicity. Further Histopathology Investigation needs to be performed to confirm exact mode of action of Triton X-100. There was a significant decrease in Body weight and feed consumption after Triton X-100 injection. All animals that were administered plant extract showed signs of piloerection and itching. *Anethum sowa* might give an improved protective activity at higher doses or alternative if administered for more than six days. Further studies need to be conducted to explore the Hypolipidemic potential of *Anethum sowa*.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Assessment of *In Vitro* Anti-Malarial Potential of Extracts of *Carica papaya*, *Swertia chirata* and *Punica granatum*

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Mira Ramaiya¹ and Abhay Chowdhary¹

ABSTRACT

Introduction: Malaria is one of the most prevalent diseases of the world. The World Health Organization (WHO) estimates 500 million cases and 2.7 million deaths occur from malaria every year. Development of new therapeutic approaches to control malaria is very much needed, since resistance of parasite to different anti-malarials is fast developing. Keeping this in mind we evaluated the *in vitro* anti-plasmodial activity of three plants against *Plasmodium berghei*.

Materials and Methods: *Plasmodium berghei* ANKA strain was used for the study. Leaf extracts of *Carica papaya* and *Swertia chirata* and rind extract of *Punica granatum* were tested for anti-malarial activities using WHO defined protocols. *In vitro* median cytotoxic concentration was determined colorimetrically using the Vero cell lines by MTT assay.

Results: Experimental values of methanolic extract of rind of *Punica granatum* was found to be most effective of the three plants (IC_{50} = 69.68 μ g/mL) followed by *Swertia chirata* leaves (IC_{50} = 72.22 μ g/mL) and leaves of *Carica papaya* (IC_{50} = 74.73 μ g/mL).

Conclusion: The results of the preliminary studies carried out with the above mentioned plant extracts are encouraging. Therefore the active constituents from these extracts could be isolated, identified and characterized for malaria therapy.

Keywords: *Plasmodium berghei*, Anti-malarial, Anti-plasmodial, *Carica papaya*, *Swertia chirata*, *Punica granatum*

INTRODUCTION

WHO estimates that 500 million cases and 2.7 million deaths occur from malaria every year. Over 2000 million people in about 95 countries of 3 continents (Asia, Africa and America) are at risk of getting malaria^[1]. Herbal remedies for malaria are already in popular use. The first antimalarial developed was Quinine, prepared from Cinchona bark, and Artemisinin, obtained from *Artemisia annua*. Synthetic anti-malarials developed later are Chloroquine, Primaquine, Proguanil, Pyrimethamine, Mefloquine, etc. Considering the fact that the parasite is continuously developing resistance to existing drugs, lack of available vaccines and adverse reactions to synthetic drugs there is need to develop novel anti-malarial drugs^[2]. Tradition of using plant products to treat febrile illnesses has grown over many generations. Therefore plant product with specific clinical activity can be the starting point for new anti-malarial drug discovery. Plants are rich source of bioactive compounds; simple chemical modifications of plant product can lead to a series of highly potent anti-malarials that can play an important role in the treatment of malaria.

In the present study plants selection was based upon the ethno botanical approach. Previous studies have shown anti-malarial activity of petroleum extract of rind (fruit-peel) of papaya (*Carica papaya*), root extract of Chirata (*Swertia chirata*) and fruit of pomegranate (*Punica granatum*)^[3-5]. The present approach was to evaluate the plant part which has not been reported earlier for anti-malarial activity.

MATERIALS AND METHODS

Plant collection: The leaves of *Carica papaya* (papaya) a tropical plant grown extensively in India and *Swertia chirata*, a native Indian medicinal plant were obtained from Veer Jijamata Udyan Municipal Nursery (Byculla, Mumbai). The fruit of *P. Granatum* (pomegranate) was obtained from local market.

Plant extraction: Methanolic extracts of the plant material were obtained using a Soxhlet apparatus. The solvent fraction was evaporated using a vacuum rotary evaporator.

Cytotoxicity assay: The cytotoxicity of the plant extracts was determined using Vero cell lines by MTT assay to assess their *in vitro* toxicity^[6]

***In vitro* culture of *Plasmodium berghei*:** *Plasmodium berghei* ANKA strain was obtained from Tata Institute of Fundamental Research (TIFR), Mumbai and cultured as described by Trager-Jensen, in the Candle Jar desiccators^[7]. The parasites were grown in rodent RBCs. The culture medium RPMI-1640 (Gibco-BRL) composed of 35 mM HEPES, 4 g/L glucose, 25 mM of Sodium bicarbonate buffer (2.1%), 1 μ g/mL gentamicin, and was supplemented with 0.5% AlbuMix II (Gibco-BRL)^[8].

Parasitaemia

In vitro Anti malarial Activity:

Preparation of plant extract: All the stocks of plant extracts were freshly prepared in DMSO and filter sterilized by passing through 0.22 μ m

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Millipore membrane. All subsequent dilutions of plant extracts and drug were made in Complete RPMI-1640 medium.

Final concentration of drug in the plate: Parasite culture was synchronized at the ring stage with D-Sorbitol treatment before using in the experiment^[9]. A parasitized (parasitemia 0.5 to 1.0%) suspension with 5–7% hematocrite was prepared in complete culture medium. The suspension of P-RBC (parasitized RBC) was added (180 µl/well) in flat bottom 96 well titre plate (Tarsons, India). The parasites were exposed to different concentrations of plant extract. Each concentration was tested in triplicates. The final volume per well was adjusted to 200 µL/well. The extract concentrations tested were ranged from 30–500 µg/ml. The plates were kept in candle jar desiccators & incubated at 37°C. Medium was replaced after 24 hours in experimental and control wells and placed back in the candle jar at 37° C for another 24 hrs, after which a thin blood smear was prepared. Percent parasitaemia was then calculated to estimate percent inhibition for individual drugs. Using the formula given below

Average % Inhibition = (Mean % parasitemia in Control – Mean % parasitemia in Test) × 100/ Mean % parasitemia in Control

Sr. No.	Name & Part of the plant	% Inhibition of parasitemia at concentrations in µg/mL						IC ₅₀ (µg/mL)
		30	60	120	240	360	500	
1	<i>Punica granatum</i> (rind of fruit)	31.12	47.88	64.93	69.66	83.85	91.08	69.68
2	<i>Carica papaya</i> (leaves)	28.61	46.55	61.96	71.93	81.19	90.93	74.73
3	<i>Swertia Chirata</i> (leaves)	25.91	44.48	57.29	75.15	86.7	92.63	72.22

Table 1: IC₅₀ values of different plant extracts in comparison with standard control

DISCUSSION

Plants have invariably been a rich source for new drugs and some anti-malarial drugs in use today (quinine and artemisinin) were either obtained from plants or developed using their chemical structure as templates. Experimental values of methanolic extract of rind of *Punica granatum* was found to be most effective of the three plants Extracts of large number of plants species, including many that are used in traditional medicines have been evaluated for *in vitro* anti-plasmodial activity^[10].

These extracts however, must be subjected to further specific investigations by isolating the compound showing anti-malarial activity. Fractionation and identification of the active compound may help us in

RESULT

Cytotoxic Evaluation of The Plant Extracts: Cytotoxic assay performed to check CC₅₀ which was approximately in the range of 2000–3000 µg mL for all three plants (Figure 1). On this basis the concentrations below 500 µg/mL were selected for *in vitro* anti-malarial activity.

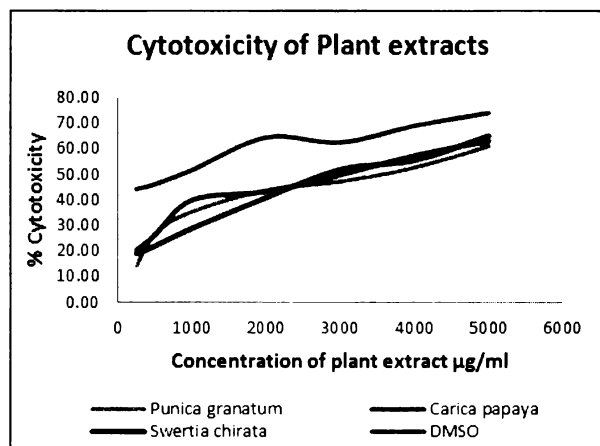


Figure 1: Percent cytotoxicity of various plants extracts

Anti-Malarial Activity of The Plant Extracts:

Anti-malarial activity of the plant extract in terms of IC₅₀ values are presented in Table 1.

increasing the efficacy and activity and decreasing its toxicity levels. A significant global network and research initiative on traditional antimalarial methods was initiated by Dr. G. Bodeker^[11]. It will be interesting in studying the synergistic effect of these plant extracts against resistant species. Thus the treasure of knowledge which imbibes in our traditional systems of medicine must be complemented with contemporary scientific analysis.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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Pharmacological Potential of *Tylophora Indica* (Antamul) Callus

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ABSTRACT

INTRODUCTION: Antioxidant compounds in food play an important role as a health protecting factor. The main ability of an antioxidant compound is its ability to trap free radical, which help to cure many diseases including asthma. *Tylophora indica* is an anti-asthmatic plant has been reported as an antioxidant agent. To evaluate the cytotoxicity as well as antioxidant activities in callus of *Tylophora indica* offers an alternative mean to produce pharmacological active compounds.

MATERIALS AND METHODS: In the present study, a method to generate a callus of the plant *Tylophora indica* was standardized. The cytotoxic activity of methanolic and water extracts of six-week-old callus of were examined on Vero cell line using the MTT cell proliferation assay. Reduction of the viability of Vero cells in the presence and absence of the extracts was evaluated. The antioxidant potential of the callus was carried out using DPPH radical scavenging activity.

RESULTS: Murashige-Skoog growth medium containing 2 ppm of Naphthalene acetic acid and 0.5 ppm of 6-Benzylaminopurine was observed to be the optimum composition for the growth and maintenance of the *Tylophora* callus, as measured by the growth index (5.11 ± 0.011). The CC_{50} of aqueous extract was higher (31.83 mg/mL) than methanolic extract (25.49 mg/mL). The IC_{50} value (as an index of anti-oxidant potential) of the aqueous, methanolic extract of *Tylophora indica* and ascorbic acid (standard) was found to be at 57.97 μ g/mL, 59.45 μ g/mL and 41.51 μ g/mL respectively.

CONCLUSION: *Tylophora indica* has been documented in Ayurveda as an antioxidant that can be administered to asthma patients. The present study was carried out to evaluate the antioxidant and cytotoxic activities of the callus of *Tylophora indica*.

Keywords: *Tylophora*, anti-oxidant, asthma, IC_{50}

INTRODUCTION

The plant kingdom consists of about 400,000 plant species and is a huge reservoir of bioactive molecules, many of which have yet to be explored for various pharmaceutical applications. Asthma is a frightening condition which can seriously impede one's ability to breathe. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases. The main ability of an antioxidant compound is its ability to trap free radicals. These are the vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress^[1]. Ayurveda is an example of a long standing tradition that offers a unique insight into comprehensive approach to asthma management through proper care of the respiratory tract function of the lungs. *Tylophora indica* (commonly known as Antamul), belonging to the Family *Asclepiadaceae* is a medicinal plant having antioxidant activity. It is a dark copper coloured delicate creeper found growing wild in the plains of India and other sub-tropical regions of the world. *Tylophora indica* extracts have been analysed for inflammatory as well as allergenic symptoms of asthma, providing prolonged relief to the sufferers^[2]. The anti-asthmatic activity of the plant is attributed to the presence of phenanthroindolizidine alkaloids^[3-6]. Antioxidant activity of *Tylophora*

indica leaves has been also been evaluated for anti-asthmatic principles^[7-9].

Finite natural resources fail to meet the ever increasing demand on medicinal plants. An effective alternate method is to develop tissue *in vitro* which can yield the same phytoconstituents in larger quantities. Natural sources of plants may have contamination by bacteria, virus, fungi, insects, etc. while plant tissues grown *in vitro* are free from the same. Studies relating antioxidant activity and asthma have observed a significant dependence of curing of asthma upon intake of antioxidant containing compounds^[10, 11]. Therefore the evaluation of cytotoxicity and antioxidant activity of anti-asthmatic plants is very critical, as well as important to find out an alternative and convenient source of these plants to conserve our green resources.

The tissue culture studies for callus induction were carried out by many studies, but their growth media was different from the ones used in the present study^[12, 13]. The antioxidant activity has not been reported so far in callus of *Tylophora indica*. The present study pertains to the cytotoxicity and antioxidant activities of six-week-old callus of anti-asthmatic plant *Tylophora indica* for the first time. The cytotoxicity examinations were carried out on Vero cells using the MTT assay^[14] and the DPPH method was used to examine the antioxidant activity of *Tylophora indica* callus^[15].

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MATERIALS AND METHODS

Initiation of the callus:

Leaves were collected from a single mature plant of *Tylophora indica*, which was obtained from farm house of Kelkar Institute, Mumbai. The leaves were washed with 1% Teepol detergent solution and then rinsed thoroughly in running water. Surface sterilization was performed by immersion in 0.1% aqueous mercuric chloride for 5 min. followed by five rinses in sterile double-distilled water. The leaves were then cut into 0.5 cm segments and cultured on callus induction medium aseptically. The medium used in all experiments consisted of Murashige-Skoog medium (MS), wherein basal salts were supplemented with 3% sucrose, 1% agar (both from Hi-Media, Mumbai, India) and various growth regulators (Naphthalene acetic acid, 6-Benzylaminopurine, Kinetin, 3-Indole Butyric acid) in various concentrations. The pH of the medium was adjusted to 5.8. Culture tubes were filled with 15 mL of the medium before autoclaving. The media was autoclaved at 121°C for 15 min. All culture tubes were incubated in the growth room having controlled condition of 1000 lux of light, 70% humidity and $25 \pm 1^\circ\text{C}$ temperature with 16 hr photo and 8 hr dark period. Six-week-old callus of *Tylophora indica* were harvested to examine the cytotoxicity (MTT assay)^[14] and antioxidant activity (DPPH radical scavenger method)^[15].

Preparation of Extracts:

Six-week-old callus of *Tylophora indica* was harvested and dried. The dried callus was ground into powder and exhaustively extracted using a Soxhlet's apparatus with distilled water and methanol separately. The extracts were concentrated and these crude extracts were used for further investigation for potential antioxidant properties.

Cytotoxicity Assay:

Vero cell line was trypsinised and suspended in Minimum Essential Medium (MEM) and were then seeded in 96 well tissue culture plate. Plates were incubated overnight at 37°C in humidified 5% CO₂ incubator. One Hundred microliters of two-fold dilution (75.0 mg/mL, 37.5 mg/mL, 18.75 mg/mL, 9.35 mg/mL, and 4.68 mg/mL) of extracts were added to the wells and again incubated for 10–16 hrs at 37°C in incubator. Cell control and positive control containing cells and Dimethyl Sulphoxide (DMSO) was also maintained along with the tests samples. Subsequent to this incubation, 10 µL of 5 mg/mL of MTT (3,4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent was added in

each well and incubated at 37°C for 4 hrs in CO₂ incubator. After the incubation 100 µL of DMSO was then added to each well. The well plates were then kept on shaker for 1 min. and readings were taken on an ELISA reader at 550 nm.

Antioxidant Assay:

The antioxidant activities of aqueous and methanolic extracts of *Tylophora indica* callus were determined by the DPPH free radical scavenging assay method. All the assays were carried out in triplicate and average values were considered.

The free radical scavenging capacity of the aqueous and methanolic extracts of *Tylophora indica* callus were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH solution (0.004% w/v) was prepared in 95% methanol. Extracts of *Tylophora indica* callus were mixed with 95% methanol to prepare the stock solution (0.1 mg/mL). Freshly prepared DPPH solution was added in each of these test tubes containing callus aqueous and methanolic extracts separately (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL) and after 10 min, the absorbance was taken at 517 nm. Ascorbic acid was used as a reference standard. Ninety-five percent methanol was used as blank.

Percent scavenging of the DPPH free radical was measured using the equation:

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{Absorbance of Control} - \text{Absorbance of test Sample})}{(\text{Absorbance of Control})} \times 100$$

RESULTS

Tylophora indica callus was initiated in the Murashige-Skoog basal medium with different growth factors. Growth Index as illustrated in Table 1 was used as a measure of the appropriate growth of the callus.

This Index was calculated using the formula:

$$\text{GI (Growth Index)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$$

Sr. No.	IBA (ppm)	NAA (ppm)	BAP (ppm)	Kinetin (ppm)	GI
A	-	2	0.2	-	5.69 ± 0.011
B	-	1	0.1	0.1	3.11 ± 0.052
C	-	1	0.1	0.2	3.98 ± 0.041
D	-	2	-	0.1	5.12 ± 0.032
E	0.5	-	0.2	-	2.11 ± 0.011
F	1.0	-	0.1	-	2.18 ± 0.019

Table 1: Effect of different growth hormones on growth of callus (6 weeks old) initiated from the leaf explant

Cytotoxicities of the six-week old callus extracts were estimated to study if the extracts were suitable for further evaluation. Cytotoxicity was measured by performing the cell proliferation assay using the MTT assay^[14]. The percent cytotoxicity measured is presented in Table 2, Figures 1 & 2.

Concentration	Inhibition due to Callus Extracts	
	Aqueous	Methanolic
75.0 mg/mL	92.2%	94.67%
37.5 mg/mL	93.34%	91.99%
18.75 mg/mL	5.73%	16.48%
9.35 mg/mL	0	0
4.68 mg/mL	0	0

Table 2: Percent cytotoxicity of *Tylophora indica* callus

The antioxidant activity of aqueous and methanolic extracts of *Tylophora indica* callus was determined by the DPPH free radical scavenging assay method. DPPH radical scavenging activity of aqueous, methanolic extracts of *Tylophora indica* callus and ascorbic acid are presented in Table 3.

Conc. (µg/ml)	% Inhibition by <i>T. indica</i> callus		% Inhibition by Ascorbic acid
	Aqueous Extract	Methanolic Extract	
10	19.31 ± 0.032	22.91 ± 0.142	24.73 ± 0.096
20	21.17 ± 0.121	26.98 ± 0.087	29.98 ± 0.067
30	32.82 ± 0.142	29.74 ± 0.043	32.65 ± 0.094
40	39.61 ± 0.143	31.98 ± 0.064	45.66 ± 0.064
50	41.62 ± 0.043	37.54 ± 0.095	57.87 ± 0.063
60	58.97 ± 0.085	64.072 ± 0.054	67.55 ± 0.086

Table 3: Antioxidant activity of *Tylophora Indica* callus

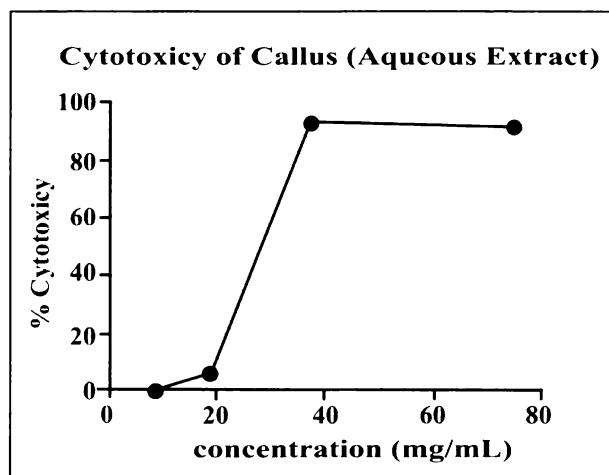


Figure 1: CC_{50} of *Tylophora indica* callus extract: 31.83 mg/mL (aqueous)

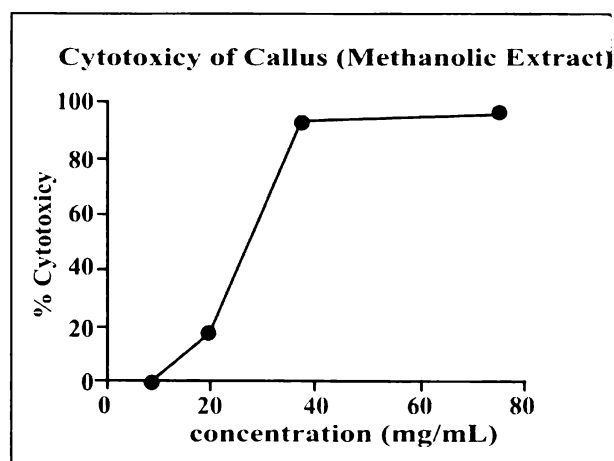


Figure 2: CC_{50} of *Tylophora indica* callus extract: 25.49 mg/mL (methanolic)

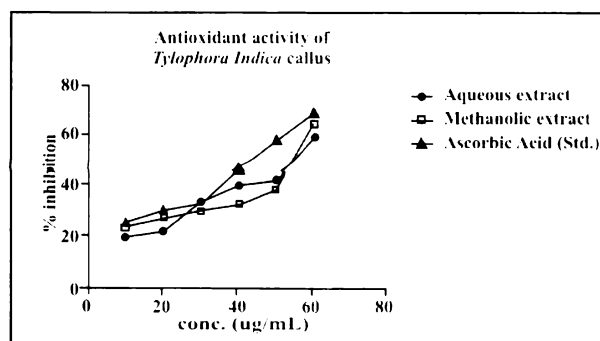


Figure 3: Antioxidant activity of *Tylophora Indica* callus as measured by DPPH radical scavenging activity

DISCUSSION

Tylophora indica is the climber plant having anti-asthmatic principle tylophorin. The flourished callus of *Tylophora indica* has been established successfully on MS medium supplemented with 2 ppm of NAA and 0.2 ppm of BAP by leaf explant. The colour of the callus was pale green and fragile in nature in all the instances. The aqueous and methanolic extracts of *Tylophora indica* were examined for cytotoxicity on Vero cells using MTT assay and antioxidant activity by using DPPH radical scavenger method.

In cytotoxicity experiment the CC_{50} of aqueous extract was maximum (31.83 mg/mL) than methanolic extract (25.49 mg/mL). The IC_{50} value of the aqueous and methanolic extract of *Tylophora indica* and ascorbic acid was found to be at 57.97 µg/mL, 59.45 µg/mL & 41.51 µg/mL respectively.

The present study shows that the aqueous extract is more potent to cure the asthmatic diseases than methanolic extract of *Tylophora indica* callus.

In conclusion, the aqueous extract of *Tylophora indica* callus can be a source of antioxidant agent. These results should be encouraging for further research work on *Tylophora indica* tissue culture for isolation of bioactive agents using phytochemical and biological investigations. Previous reports of antioxidant activities support the present study^[9]. The callus of this plant can be used as antioxidant agent so it would be useful in protecting our green cover.

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Synthesis and Biological Activity of Some Substituted 4-Thiazolidinones

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ABSTRACT

Introduction: 4-Thiazolidinone derivatives play a vital role in pharmaceutical sciences owing to their wide biological & industrial applications. The presence of chloro substituent in the phenyl ring of a molecule has been reported to increase the activity of the molecule. The recent reviews reported that benzothiazole nucleus is associated with anti-bacterial and anti-fungal activities. In the view of these efforts was made to synthesised compound for their Anti-Microbial activity.

Materials and Methods: Organic molecule contain 4-Thiazolidinones moiety synthesised in Department of Chemotherapy, Haffkine Institute, Mumbai. All synthesised compounds screen for their in vitro Anti-Microbial activity against anti-bacterial and anti-fungal strain using Tube Dilution method. The culture medium used in screening was Muller-Hinton broth.

Results: All the compounds were screened for their anti-bacterial activity by dilution against *Staphylococcus aureus* (NCTC 3750) and *Salmonella typhi* (NCTC 786) using Ampicillin (MIC 0.01 µg/mL) and Trimethoprim (MIC 0.01 g/mL) as standard drug. The compounds were screened for their anti-fungal activity by tube dilution technique against *Candida albicans* (ATCC 16404) and *Aspergillus niger* (ATCC 16404) using Miconazole (MIC 1.56 µg/mL) as standard drug. Compounds exhibited good activity against *S. aureus*, *C. albicans* and *A. niger*; however, no remarkable activity against *S. typhi* was observed. The culture medium used in screening was Muller-Hinton broth.

Conclusion: Compound having Electron Withdrawing substituent shows enhancing antimicrobial activity as compared to substituent with electron donating group.

Keywords: 4-Thiazolidinones, Benzothiazole, Anti-Microbial.

INTRODUCTION

Compounds containing 4-Thiazolidinones nucleus posses various biological activities such as anti-fungal activity^[1] and anti-inflammatory activity^[2]. 4-Thiazolidinone derivatives play a vital role in pharmaceutical sciences owing to their wide biological and industrial applications^[3]. The derivatives of 4-Thiazolidinones have also occupied a unique place in the field of medicinal chemistry due to a wide range of pharmacological activities^[4]. The presence of chloro substituent in the phenyl ring of a molecule has been reported to increase the activity of the molecule^[5].

Heterocycles bearing Thiazole, Sulphur and Nitrogen constitute the core structure of number of pharmacologically and biologically active compounds. The presence of N-C-S linkage in heterocycles has been reported to possess anti-fungal activity^[6]. Recently it is reported that benzothiazole nucleus is associated with anti-bacterial and anti-fungal activities^[7]. Therefore it was considered worthwhile to explore the synthesis of compounds built upon benzothiazole skeleton incorporating Thiazolidinone moiety with the hope of potentiating the activity of two such units in same compounds.

MATERIAL AND METHODS

All commercial solvents used in the experimental work were redistilled and dried before use. Melting points were recorded on a Thermo-nik Melting point Apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. IR spectra were recorded on a Hartmann Braun, MB-104 Series (Bomem, Quebec, Canada) in KBr Pellets. ¹H-NMR spectra have been recorded on a JEOL AL-300 FT-NMR spectrometer (300 MHz, JEOL Ltd., Tokyo, Japan), using TMS as internal standard in solvent DMSO. Elemental analysis has been carried out on a C, H, and N Elemental Analyzer (Thermo-Finnigan Flash EA 1112, Italy).

Experimental (Figure 1)

Synthesis of 6-Chloro-2-hydrazino benzothiazole (Compound 2)

Conc. HCl 4 mL was added drop wise with stirring to hydrazine hydrate 4 mL at 5°–10° C. To it ethylene glycol (16 mL) and 6-Chloro-2-amino benzothiazole (compound 1) was added and refluxed for 3 hrs. On cooling solid separates out which was filtered, washed with water and recrystallized with ethanol.

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6-Chloro benzothiazol-2-yl hydrazones (compound 3)

Compound **2** (0.1 mol; 19.95 g) and substituted aromatic benzaldehydes were dissolved in 50 mL of absolute ethanol. To this, a pinch of anhydrous ZnCl_2 was added. The reaction mixture was refluxed on a water bath for 15–18 hrs. It was cooled and poured over crushed ice; the solid thus obtained was filtered, washed with water and recrystallized from ethanol.

Synthesis of 3-(6-Chloro benzathiazol-2-ylamino)-2-(substituted-phenyl)-thiazolidin-4-one (compound 4)

A mixture of **3** (0.005 mol) and Thioglycollic acid (0.005 mol; 0.35mL) in DMF (15 mL) containing a pinch of anhydrous ZnCl_2 was refluxed for 6 hrs. The reaction mixture was cooled and poured over crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from glacial acetic acid.

IR (KBr) cm^{-1} : 3095 (CH), 1724 (C=O), 3360 (NH), 1089 (CN).

^1H NMR (DMSO): δ 3.35 (s, 2H, SCH₂), 3.51 (s, 1H, CH-Ar), 7.26-8.05(m, 9H, 8H Ar-H, 1H, NH)

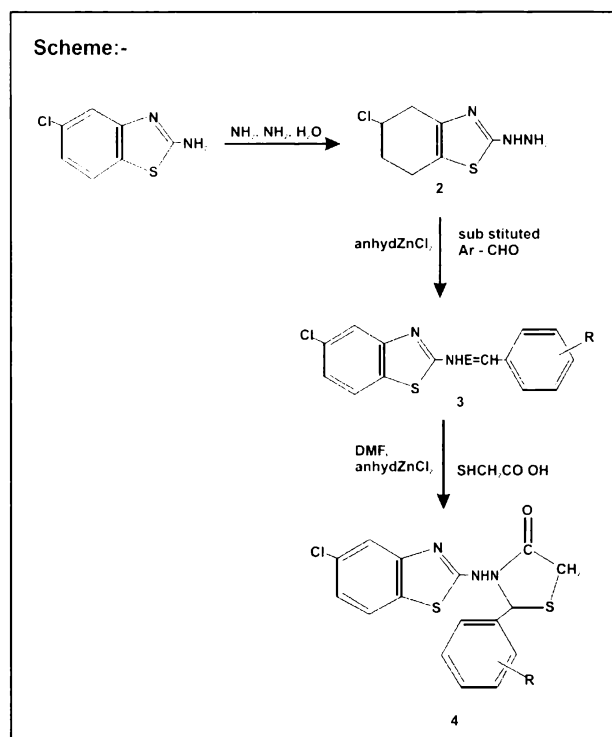


Figure 1: Schematic representation of synthesis

Anti-microbial Activity

The synthesized compounds were screened for their anti-bacterial and anti-fungal activities. The various screenings carried out include the *in vitro* study against Gram positive *Staphylococcus aureus* (ATCC 3750), Gram negative *Salmonella typhi* (NCTC 786) and fungal strains *Candida albicans* (ATCC 10233) and *Aspergillus niger* (ATCC 16404). The Minimum Inhibitory Concentration (MIC) was determined using tube dilution method according to the standard procedure [8]. DMSO was used as a solvent with appropriate controls. Ampicillin (MIC = 0.01 g/mL against gram positive *S. aureus*, Trimethoprim (MIC = 1 g/mL against gram negative *S. typhi*) were used as standard drugs for anti-bacterial screening and Miconazole was used as anti-fungal standard drug (MIC = 0.01 g/mL against *C. albicans* and *A. niger*).

RESULTS

The results of the *in vitro* antimicrobial activity are shown in Table 1. Most of the compounds showed activity against tested organisms. The compounds 4b, 4d, 4h, 4j and 4c, 4f, 4g, 4i proved to be active against *S. aureus* and *C. albicans* respectively. Further 4f, 4g, 4j were found to be active against *A. niger*. The rest of the compounds showed varying degree of activity against the test organisms.

DISCUSSION

It was found that the compounds having substituted benzaldehydes attached with electron withdrawing groups were more potent than the electron donating groups. Carboxy substituted benzaldehydes were the most potent amongst them. Presence of plain benzaldehydes also showed moderate activity. It may be concluded that chloro substituent in the phenyl ring of a molecule increases the activity of the molecule.

Comp. No	R	MIC against			
		<i>S. aureus</i> (µg/mL)	<i>S. typhi</i> (µg/mL)	<i>C. albicans</i> (µg/mL)	<i>A. niger</i> (µg/mL)
4a	H	100	200	100	200
4b	4-chloro	50	100	200	200
4c	4-carboxy	200	100	50	200
4d	4-hydroxy	50	200	100	100
4e	4-fluro	100	200	100	100
4f	2-nitro	200	200	50	50
4g	2,3-dihydroxy	100	100	50	40
4h	2,6-dichloro	50	100	100	200
4i	2,4-dimethoxy	200	100	50	100
4j	3,4,5-trimethoxy	50	100	100	40

Table 1: Antimicrobial activities of synthesized compounds against test organism (MIC)

Ampicillin (MIC 0.04 g/mL) used as standard against *S. aureus*; Trimethoprim (MIC 0.01 g/mL) used as standard against *S. typhi*; Miconazole (MIC 6.25 g/mL) as standard against *C. albicans* and *A. niger*

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

☞ – ☞

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Mosquito Larvicidal Activity of Indigenous Plant Extract against *Aedes aegypti* Larvae

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ABSTRACT

Introduction: The best way to keep the mosquitoes at check is larviciding. Biological control is an attractive alternative to chemical pesticides, particularly in view of the current restrictions on pesticide use and habitat management, and continuing problems with chemical resistance. Therefore continuous efforts are being made to look for safer alternative larvicide. In the present study the seed oils (Petroleum ether extract) of four plants, *Ricinus communis*, *Pongamia pinnata*, *Annona squamosa* and *Eclipta alba* were tested against *Aedes aegypti* (Diptera: Culicidae) mosquito larvae.

Materials and Methods: The larvicidal bioassay of the concentrated oily extracts of *Ricinus communis*, *Pongamia pinnata*, *Annona squamosa* and *Eclipta alba* all four plants were screened against early fourth instars *Aedes* larvae using WHO standard protocol. Laboratory reared *Aedes aegypti* were used for larvicidal bio-assay. Since *Ricinus communis* produced more promising activity the extract was further subjected to column chromatographic fractionation with silica gel prepared in benzene were tested for larvicidal activity.

Results: Of the four plant extracts examined, extracts of *R. communis* and *P. pinnata* demonstrated larvicidal activity with LC₅₀ value 0.073% and 0.138% respectively. Column chromatographic fraction of *Ricinus communis* with Fraction B (benzene: methanol band 95:5) produced excellent larvicidal activity.

Conclusion: In conclusion, Seed oil extract of *R. communis* and *P. pinnata* showed potent larvicidal activity. Chromatographic fractions of *Ricinus communis*, FB (benzene: methanol band (95:5) possessed larvicidal activity at much lower concentration than the original seed extract of this plant.

Keywords: *Aedes aegypti* Mosquito, Larvicidal activity, *Ricinus communis*, *Pongamia pinnata*

INTRODUCTION

Mosquitoes are important pests that affect human health and well-being throughout the world. Mosquitoes in the larval stage are attractive targets for pesticides because they breed in water and, thus, are easy to deal with them in this habitat^[1]. A variety of synthetic insecticides are used for chemical control of mosquito larvae.

Although these insecticides are effective they pose serious public health and environmental hazards besides they develop insecticide resistance and undesirable effect on non-target organism^[2]. Biological control is an attractive alternative to chemical pesticides. Therefore, continued, efforts are being made to look for safer, alternative mosquito larvicides. In one such study at Haffkine Institute, *Adenanthera pavonina* seed extract was found to have excellent mosquito Larvicidal activity^[3, 4]. Encouraged by these findings four more plants were screened against *Aedes aegypti* mosquito larvae.

MATERIALS AND METHODS

The larvicidal bioassay was carried out using WHO standard protocol^[5]. Laboratory reared *Aedes aegypti* were used for larvicidal bioassay under laboratory conditions at 25–30° C and 75–80% relative humidity.

Seeds of “Karanja” (*Pongamia pinnata*), “Sithaphal” (*Annona squamosa*), “Bhringaraj” (*Eclipta alba*) and ‘Castor’ (*Ricinus communis*) were dried in the shade and powdered to uniform mesh using Willey Mills. The material was then extracted in a soxhlet extraction apparatus for 24 hrs with Petroleum ether (40–60° C) and made solvent free by heating on controlled temperature water bath. Concentrated oily extracts were then screened against fourth instars *Aedes* larvae. Different concentrations were prepared in beakers containing 250 mL dechlorinated tap water. Twenty-five fourth instar larvae (laboratory reared) were released in each beaker for 24 hrs. Doses range was fixed by conducting a non-replicated pilot study with a wide range of concentrations Larvicidal assay was carried out for each extract separately. Three replicates were kept for each concentration including untreated control. No food was added in the beakers as per WHO norms. Mortality was recorded after 24 hrs of treatment by counting dead and moribund larvae. Pupated larvae were discarded. Corrected mortality was calculated by Abbot’s formula^[6].

Since Castor seed oil showed more promising activity, it was subjected to column chromatographic fractions with silica gel (60–130 mesh) column (42 cm × 5 cm) prepared in benzene. The column speed was 20 drops per min. Elution was carried out in

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the same solvent (benzene) and the following elutes were collected (Table 1). These fractions were tested for larvicidal activity as per above procedure.

Sr. No	Fraction	Solvent combination
1	FA	Benzene
2	FB	benzene: methanol band (95:5)
3	FC	benzene: methanol (1:1)
4	FD	methanol

Table 1: column chromatographic fractionation of *R. communis* seed oil

RESULTS

Of the four plant extracts examined, only extracts *R. communis* and *P. pinnata* demonstrated larvicidal activity (Table 1) The LC_{50} value of *R. communis* and *P. pinnata* was observed to be 0.073% and 0.138% respectively.

As *R. communis* showed excellent larvicidal activity, a further examination of fractions of the extract was undertaken. It was observed that only one fraction FB (benzene: methanol band ; 95:5) possessed larvicidal activity. The LC_{50} value of this fraction was 0.005% (Table 2).

Plant	Larval mortality after 24 hr (%)*							LC ₅₀ (%)
	Per cent concentration							
	0.005	0.01	0.02	0.04	0.08	0.16	0.32	
<i>Ricinus communis</i>	0.00	23.65 ± 1.33	41.43 ± 0.95	59.66 ± 1.32	72.32 ± 0.94	90.18 ± 0.56	100.00	0.073
<i>Pongamia pinnata</i>	0.00	8.25 ± 0.89	20.76 ± 1.17	38.33 ± 1.43	45.66 ± 1.15	61.26 ± 1.23	91.07 ± 1.56	0.138
<i>Anonna squamosa</i>	0.00	0.00	0.00	02.27 ± 1.08	19.59 ± 1.56	24.66 ± 1.19	36.98 ± 1.02	0.406
<i>Eclipta alba</i>	0.00	0.00	3.36 ± 1.16	11.39 ± 1.12	27.28 ± 1.09	33.22 ± 1.06	48.28 ± 0.83	0.301

Table 2: Percentage larval mortality of *Aedes aegypti* for different concentrations of seed oil extract of four different plants *Average % mortality of three replicates (mean ± SD)

LC_{50} = Lethal Concentration that kills 50% of the exposed larvae after 24 hr.

Concentration of fraction FB (%)	% Larval mortality after 24 hr*
0.004	29.0 ± 1.29
0.008	50.0 ± 1.01
0.016	77.0 ± 0.09
0.032	98.0 ± 1.23
0.064	100.00
Control	0.00
LC_{50}	0.005%

Table 3: Larvicidal activity of fraction FB of *Ricinus communis* seed oil extract

*Average mortality (Mean ± SD)

LC_{50} = Concentration required to kill 50% test population

DISCUSSION

Plants could be an alternative source for mosquito larvicide because they constitute a potential source of bio-active compounds and generally free from harmful effects^[7].

Out of the four different seed oil extract tested, oil of *Pongamia pinnata*, and *Ricinus communis* showed good larvicidal activity. Since *Ricinus communis* revealed larvicidal activity at much lower

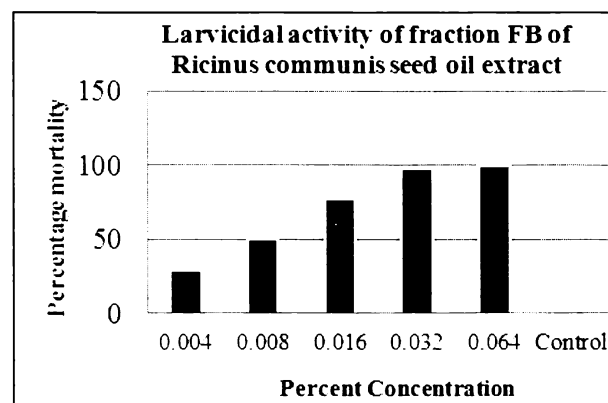


Figure 1: Larvicidal activity of fraction FB of *Ricinus communis* seed oil extract

concentration (LC_{50} : 0.073%) chromatographic fractionation was carried out. fraction FB (benzene: methanol band (95:5) came out with excellent activity which was much lower than the original seed extract of this plant. Previous studies reported crude oil extract of neem and Karanja effective against *Cx. Quinquifasciatus* larvae^[8-10]. Reports on Larvicidal activity of neem and karanja oil cakes were also found effective against all three species of mosquito larvae^[11].

the same solvent (benzene) and the following elutes were collected (Table 1). These fractions were tested for larvicidal activity as per above procedure.

Sr. No	Fraction	Solvent combination
1	FA	Benzene
2	FB	benzene: methanol band (95:5)
3	FC	benzene: methanol (1:1)
4	FD	methanol

Table 1: column chromatographic fractionation of *R. communis* seed oil

RESULTS

Of the four plant extracts examined, only extracts of *R. communis* and *P. pinnata* demonstrated larvicidal activity (Table 1) The LC_{50} value of *R. communis* and *P. pinnata* was observed to be 0.073% and 0.138% respectively.

As *R. communis* showed excellent larvicidal activity, a further examination of fractions of the extract was undertaken. It was observed that only one fraction FB (benzene: methanol band ; 95:5) possessed larvicidal activity. The LC_{50} value of this fraction was 0.005% (Table 2).

Plant	Larval mortality after 24 hr (%)*							LC ₅₀ (%)
	Per cent concentration							
	0.005	0.01	0.02	0.04	0.08	0.16	0.32	
<i>Ricinus communis</i>	0.00	23.65 ± 1.33	41.43 ± 0.95	59.66 ± 1.32	72.32 ± 0.94	90.18 ± 0.56	100.00	0.073
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<i>Eclipta alba</i>	0.00	0.00	3.36 ± 1.16	11.39 ± 1.12	27.28 ± 1.09	33.22 ± 1.06	48.28 ± 0.83	0.301

Table 2: Percentage larval mortality of *Aedes aegypti* for different concentrations of seed oil extract of four different plants *Average % mortality of three replicates (mean ± SD)

LC_{50} = Lethal Concentration that kills 50% of the exposed larvae after 24 hr.

Concentration of fraction FB (%)	% Larval mortality after 24 hr*
0.004	29.0 ± 1.29
0.008	50.0 ± 1.01
0.016	77.0 ± 0.09
0.032	98.0 ± 1.23
0.064	100.00
Control	0.00
LC_{50}	0.005%

Table 3: Larvicidal activity of fraction FB of *Ricinus communis* seed oil extract

*Average mortality (Mean ± SD)

LC_{50} = Concentration required to kill 50% test population

DISCUSSION

Plants could be an alternative source for mosquito larvicide because they constitute a potential source of bio-active compounds and generally free from harmful effects^[7].

Out of the four different seed oil extract tested, oil of *Pongamia pinnata*, and *Ricinus communis* showed good larvicidal activity. Since *Ricinus communis* revealed larvicidal activity at much lower

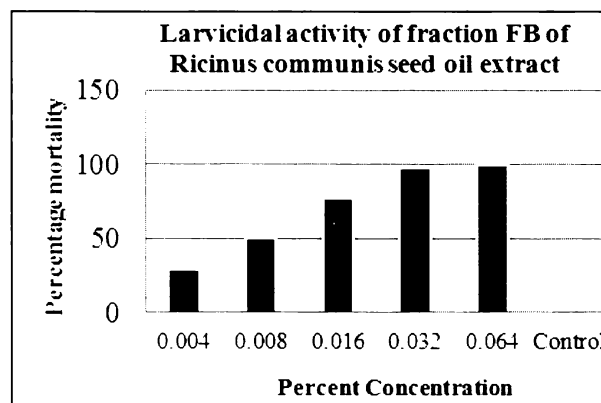


Figure 1: Larvicidal activity of fraction FB of *Ricinus communis* seed oil extract

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Since both “Castor” and “karanja” plants are widely distributed, further identification of active compounds, toxicity and field trials are needed to recommend the active fraction of both these plant for larvicidal use.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.



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Surveillance of Plague in Rodents from Mumbai Region

K R Pawar^{1,2}, P Ghuge², Vishal B Wagh¹ and Abhay S Chowdhary^{1*}

ABSTRACT

Plague is one of the most severe bacterial infections possessing potentially deadly effect to animals as well as humans. This infectious disease is caused by the enterobacteria *Yersinia pestis*. In India, the first outbreak of plague occurred in the year 1896. In Maharashtra State, a Plague surveillance unit consisting of Municipal Rat Destruction Establishment (MRDE) was setup at Haffkine Institute, Mumbai in the year 1953 and since then regular Plague surveys are carried out in Plague prone areas. In 1994, a plague outbreak occurred in the Beed district region of Maharashtra followed by an outbreak in Mumbai region. Rodents captured by BrihanMumbai Municipal Corporation (BMC) are brought to this centre for identification and analysis. During January to December 2010, 3,27,529 rodents were analysed at Haffkine institute. In all, seven species of rodents were identified and out of which 5,919 samples were selected to check the presence of fleas (*Xenopsylla cheopis*)—the vector of plague bacteria (*Yersinia pestis*). The epidemiological information collected during this study was helpful in providing a clear picture of different rodent species present in different areas for implementing effective and timely rodent control measures.

Keywords: Plague, *Xenopsylla cheopis*, *Yersinia pestis*, Rodents, Fleas

INTRODUCTION

Plague, an infectious disease of animals and human beings is transmitted to humans and animals by flea bite, infected with *Y. Pestis*. Rodents are the main carrier of fleas^[1]. World Health Organization (WHO) has reported 38,310 cases of plague with 2845 deaths in the world from 1989 through 2003 with majority of plague cases clustered in 25 countries in Africa and Asia^[2, 3]. Approximately 3,000 human cases occur worldwide annually, with 12 to 15 cases being reported each year in the Western United States^[4]. It may evolve into a highly infectious pneumonic form that can spread from person to person through airborne droplets^[5]. Sporadic cases in humans as well as outbreaks have been reported every year^[6].

Rodents are the largest order of mammals, i.e. around 40% mammalian species belonging to the Rodentia family^[7]. There are approximately 4,000 rodent species classified on the basis of their anatomy into three well-defined groups, or suborders, and more than 30 families. Increasing number of rodents can be of concern since they are the carriers of various pathogens causing life threatening diseases in humans and animals. Plague is primarily a disease of rodents, which inhabit the natural foci where ecological conditions ensure the persistence of the pathogen for considerable period.

In this epidemiological study, we screened wild rodents which are a reservoir of plague infection for the presence of *Y. Pestis* and identified different species of rodents prevalent in different areas of Mumbai region.

MATERIALS AND METHODS

Collection of rodents

The rodents were captured from different wards in and around Mumbai region by rat killers appointed by BMC during January to December 2010.

Identification and Analysis of sample

Rat killers killed the wild rats and brought them to Municipal Rat Destruction Establishment (MRDE) at the Haffkine Institute, where they were identified and analysed for the presence of fleas and the plague bacteria *Y. Pestis*. Rats were dissected and smears were prepared from liver and spleen and stained with Wayson stain to observe the presence of bacteria *Y. pestis*^[8] as the reference confirmatory test recommended by WHO^[5].

RESULTS

Rodent collection and Identification

A total of 3,27,529 rodents were captured during the period of one year from different representative wards of Mumbai region. Seven different species were identified viz; *Bandicota bengalensis* (1,55,567), *Rattus rattus rufescence* (30,872), *Rattus norvegicus* (26,096), *Sancus caeruleus* (1,048), *Rattus rattus wroughtoni* (722), *Mus musculus* (547) and *Bandicota indica gentiana* (281). The total numbers of rodents captured and identified during year 2010 are shown in Figure 1. Out of the total number captured, 1,12,530 rodents remained unidentified due to deformed and distorted condition.

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²Municipal Rat Destruction Establishment (MRDE), Haffkine institute, Mumbai, India

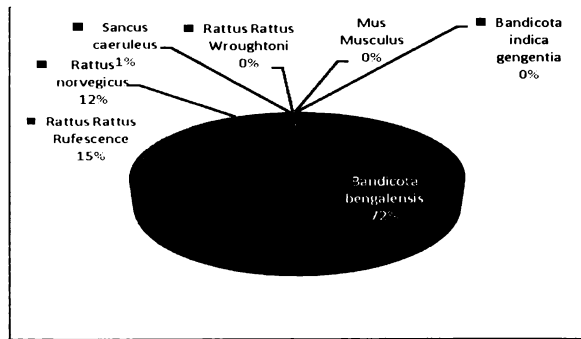


Figure 1: Total number of rodents captured during the year 2010

Of all the rodent species collected, *Bandicota bengalensis* was found to be most abundant. Following *Bandicota bengalensis*, *Rattus rattus rufescence* and *Rattus norvegicus* were found in huge numbers. Abundant presence of *Bandicota bengalensis* is probably due to their high reproductive potential and change of habitual habitat. The rare presence of *Rattus Rattus Wroughtoni*, *Bandicota indica gengantia*, *Mus Musculus* may be attributed to a change in their habitat due to the rampant development and construction in city. Highest numbers of rodent were captured in the month of June and December as shown in Figure 2.

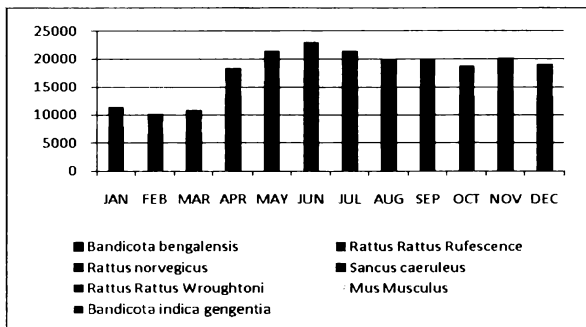


Figure 2: Month-wise distribution of rodents collected

Analyses of samples for the presence of fleas/*Y. pestis*

A total of 5,919 samples were tested for the presence of fleas and *Y. Pestis* and were found to be negative for both. (Figure 3)

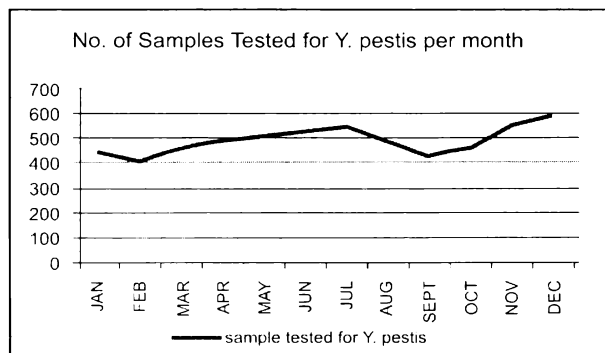


Figure 3: No. of samples tested for *Y. pestis* per month

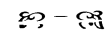
DISCUSSION

Regular survey of rodents is important to control any further outbreak of plague. Humans acquire this Zoonotic infection when animal fleas aberrantly bite them. Most infected fleas come from the domestic black rat *Rattus rattus* or the brown sewer rat *Rattus norvegicus* [8].

Even though there have been reports of a high infection of *Y. pestis* to *Bandicota bengalensis*, there is no incidence of infection with *Y. pestis* found in the rodents analysed at the Haffkine Institute during the aforesaid period. Proper and regular surveillance of plague endemic areas is one of the most important parameter for the detection of wild plague foci. This would help in undertaking systematic preventive measures against any outbreak of plague

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.



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A Case of Giant Hairy Pigmented Nevus over Upper Limb in a Young Female Patient

G R Ekbote^{1*}, P B Sahasrabudhe², Nikhil Panse³, S K Waje⁴

ABSTRACT

Excision of a congenital giant nevus is advised due to the possibility of it degenerating into a malignant melanoma or for aesthetic concerns. Tissue expansion, full thickness graft and thick split thickness graft are the options available. We present a two staged thick split thickness skin graft technique to treat a case of circumferential nevus from the midarm throughout the upper extremity distally excluding distal 5 cms. of the forearm. The affected skin was approximately 5% of the total body surface area. The patient underwent two operations and were completed over a 2-months period. A good aesthetic and functional outcome in the left upper extremity was achieved with a satisfied patient.

INTRODUCTION

Giant hairy nevus over the upper limb is a unique form of congenital nevus, fortunately rare and typically hair bearing. If is large it is disfiguring and can cause psychological disturbance. These lesions have a relatively high risk of becoming malignant. These lesions, when large, make a formidable undertaking in view of lack of suitable donor sites and multiple procedures involved in its treatment. Giant hairy nevus of the upper limb is a particular challenge to a plastic surgeon not only because of a very high standard of skill required but also for the patient and parental concern for cosmetic results. In India and in developing countries if it is present in girls it can become an obstacle in their marriage. This paper presents how we managed a giant hairy nevus over left upper limb.

CASE REPORT

A 14 year old girl, a student of 9th Std. residing at Ratnagiri, Maharashtra Presented to the OPD at Sasoon Hospital, Pune with a giant pigmented hairy nevus over the left upper limb. It was present since birth with increase in pigmentation gradually. No c/o pain or itching. Lesion involved the left forearm over flexor & extensor aspect excluding distal 5 cms. Its proximal extent is up to midarm (Fig. 1). The lesion measured about 25 cms. In length and was circumferential in nature. The child was a victim of ridicule at school and was brought for management of this major cosmetic defect by her parents. This patient was referred to Plastic Surgery. The child was planned for staged surgical management. Full thickness excision of lesion was done in staged manner first over the flexor aspect and Skin cover was given by thick split-thickness

skin graft harvested from left thigh. Two month later procedure was repeated for extensor aspect. On discharge patient was advised pressure garment, coconut oil massage for both recipient and donor site. It was also advised physiotherapy and follow up regularly. At one year follow up graft take was in good condition with good color match, contour and cosmetic appearance. Donor site healed without any hypertrophy or pigmentation. Patient was satisfied as school ridicule stopped. She also started using half sleeves.

DISCUSSION

Congenital pigmented nevi are present in approximately 2 to 3% of neonates. Fewer than 10% of these lesions are larger than 3 or 4 cms a size cut-off below which the designation small is usually given. Large and giant are debatable and confusing terms. A review of literature found that the definition of 'Giant' was variable. Persl¹ had defined 'giant' as palm size on the face and twice palm size elsewhere. While some authors felt that an area of more than 144 square inches was 'giant'^[2], others considered a lesion that could not be completely excised and closed primarily as 'giant'^[3]. It is now well established that giant congenital nevi and malignant melanoma are associated, despite the fact that the magnitude of risk of malignant transformation is still the subject of wide controversy. Divergent figures range from 1.8% to up to 45%. A recent review^[4] on the subject have calculated an 8.52% incidence of melanoma developing within Nevi larger than 2% of the total body surface during the first 15 years of life. Despite the controversy, many clinicians agree that prophylactic excision of all giant and large hairy nevi is indicated^[5].

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Figure 1: Giant hairy (shaved) nevus present over left upper limb

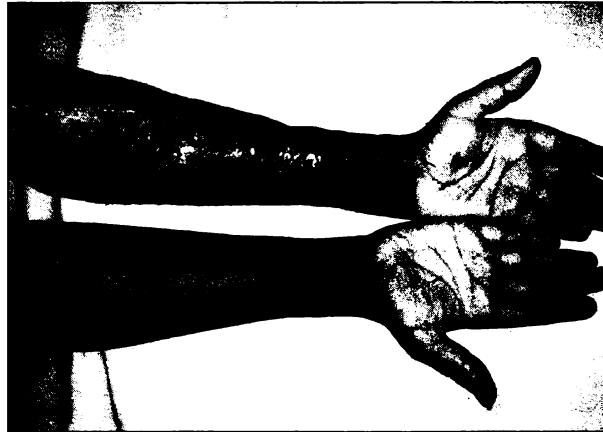


Figure 2: Post-operative (after 1 year)

The opinion regarding complete prophylactic excision of all giant nevi should be accomplished in infancy and early childhood is well supported in literature^[6] by the evidence that 60% of the malignancies developing in these lesions present in early childhood. In one series, 10 to 20 malignant melanomas arising in giant nevi were diagnosed before 3 years of age^[7]. In addition, concerns about lack of parameters to predict as to which nevi are susceptible to malignant transformation, compounded with the fact that the diagnosis gets delayed and the course in case of a malignant change is invariably fatal are reasonable^[5]. A recent review^[8] has concluded that an early aggressive approach to these lesions is responsible for the low risk of malignant melanoma reported in various series. Surgical excision results in the debulking of tissue at risk for the development of malignant melanoma.

Agreeing that early prophylactic excision is indicated, but actually it has posed a major

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KNOW YOUR SCIENTIST: MAJOR GENERAL SIR SAHIB SINGH SOKHEY



Major General Sir Sahib Singh Sokhey

Born: 15 December 1887; Amritsar; Died: 23 October 1971

Major General Sir Sahib Singh Sokhey—the first Indian Director of this Institute, from 1932 to 1949 has been a guiding force for Haffkine Institute, the presence of which is felt even today. He joined the Haffkine Institute on 18 August, 1925 as Assistant Director in Biochemistry Department. During this time Major Gen. Sokhey's dynamic leadership and dedication to science shaped the development of the Institute which flourished and with newer activities in all fronts.

He was a fellow of the Indian Academy of Sciences, National Institute of Sciences of India (New Indian National Science Academy) and Rockefeller Foundations.

Born in Amritsar on 15th December, 1887, Major General Sir Sahib Singh Sokhey joined the Indian Medical Service in 1913 and served in World War-I from 1915-21. He was awarded the Rockefeller Fellowship from 1923-25.

He was educated at Government College—Lahore, Edinburgh University, London Hospital Medical College, Trinity College—Cambridge, John Hopkins University, Harvard University and Toronto University.

Haffkine Institute owes its existence today to Major General Sir Sahib Singh Sokhey. On 26 October 1932, when he took over as the Director of the Institute he was asked to consider increasing the price of Plague Vaccine or prepare for winding up the Institute. Major Gen. Sokhey, however, recommended the opposite i.e. reducing the price of vaccine and expansion of the Institute's activities. He wrote to the government that reducing the inputs for research is not economy, whereas work aimed at improving the health of the people would bring in greater money returns and in

the long run is economical in itself.

He was first to establish the normal values of analysis of constituents of blood and urine among Indian population. He also studied the factors for growth of *Y. pestis* and developed a protein free medium based on acid hydrolysate of caesin for preparing plague vaccine. Similar animal protein free medium was developed later for cholera vaccine preparation. He developed a method of assay of plague vaccine, which is now used as standard method by testing laboratories. He also standardized the methods for assay of cholera and typhoid vaccines. Thus, he brought about many improvements in bacterial vaccine production.

Major Gen. Sokhey had to his credit held a member of distinguished appointments; he left the Institute in 1949 to take over as the Assistant Director General of World Health Organization (1950-52). He was later nominated as Member of the Rajya Sabha (1952-53). He was awarded the coveted Stalin Peace Prize in 1953. During his tenure at Haffkine, he was knighted by the Queen of England.

He has published a number of scientific papers in various science journals.

Major General Sir Sahib Singh Sokhey left for heavenly abode on 23 October 1971. The imposing personality of Major Gen. Sokhey shall not be easily forgotten, his zeal and increasing efforts towards the advancement of science make him stand out among all. Major Gen. Sokhey's life and work will definitely make a number of audiences to go back to their work benches with renewed interest. Major General Sir Sahib Singh Sokhey has been a guiding force for Haffkine Institute and shall continue to be so.

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