Original Article

An observation on direct changes in *Aedes albopictus* midgut cells by *Rhus tox* 6C in relation to dengue virus infection

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Abstract

Background and Objectives: In mosquito vectors, dengue virus (DENV) invasion occurs through midgut cells, but available mosquito cell lines for *in vitro* study of DENV are prepared from eggs or larvae, which are not appropriate models, to study its infectivity. Hence, we developed a new primary cell culture, from *Aedes albopictus* mosquito midgut, and standardized it for *in vitro* study of DENV, with an aim to find out any possible role of homoeopathic medicines, in preventing or reducing DENV invasiveness in these midgut cells. This midgut primary cell culture demonstrated prominent cytopathic effects on infection with wild DENV isolated from dengue-infected patients in viremic phase. **Materials and Methods:** In this paper, we observed the direct effect of homoeopathic medicine *Rhus toxicodendron* 6C *(Rhus tox* 6C) (ultra dilution of 10^{-12}) on this primary cell culture, to find out significant changes, to be used as baseline data in future experiments to observe possible role of *Rhus tox* 6C against DENV infection in these cells. Hence, these direct changes may be a prerequisite for the action of this medicine against DENV invasion; as this is one of common repertoire homoeopathic medicines used against dengue fever. **Conclusion and Discussion:** In our experiments, we found that *Rhus tox* 6C could increase cell size and help organization of cells on the solid surface as observed under scanning electron microscope although the total number of cells was decreased. Moreover, *Rhus tox* 6C treated cells were healthier as indicated by less number of deformed, clump, and diploform cells.

Key words: *Aedes albopictus* midgut cell line, Arbovirus, *Rhus tox* 6C

Introduction

Among the several life-threatening arboviral diseases, of the tropical and subtropical countries, the most common and fatal disease is dengue fever caused by an 11 kDa, positive-stranded RNA virus called dengue virus (DENV).[1] This *Flaviviridae* virus infection is the cause of about 20,000 annual deaths[2] and about 500,000 cases of severe dengue infections - dengue haemorrhagic fever/dengue shock syndrome. Over the past 50 years, the incidence of DENV infection has increased to 30-fold, and according to the WHO, about 50100 million people are infected with DENV each year, in over 100 countries worldwide.[3] The four genotypes (DENV 14) of this virus are transmitted by mosquitoes of *Aedes* sp., mainly *Aedes aegypti* and *Aedes albopictus.* The main clinical presentations of dengue fever are pyrexia, joint pain, skin rashes, headache, muscle pain, and anorexia.[4]

Although being a severe threat to human life, the treatment modality of dengue fever is a general one; without any specific antiviral agent, to cure the disease, and in spite of continuous trials on dengue fever vaccines, no official vaccine program has been started in any of the affected countries so far. Furthermore, except recently developed AG 129 mice (129 Sv mice deficient in alpha, beta, and gamma interferon receptors), absence of easily available proper animal model makes it more difficult, to study the pathogenesis of DENV[5] Homoeopathic medicines such as *Rhus tox; Eupatorium perfoliatum; Belladonna*; *Arsenicum album*; *Bryonia* and many others in different potencies are being used to treat DENV infected people, in different parts of India and other countries such as Brazil, Central America, Thailand, Sri Lanka,

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Pakistan and Cuba[6,7] Thus, it appears essential to study the detailed effects of these medicines at molecular levels, on *in vitro* cell lines and in animal models *in vivo*. Hence, we have developed a protocol, which will be followed in several stages, that involves preparation of primary cell culture from midgut of *A. albopictus*, followed by application of homoeopathic medicines on these cells, and subsequent studies of the effects of homoeopathic medicines on these cells, after challenging with DENV. In this paper presents preliminary results of the first part of this protocol, where we observed a direct effect of a homoeopathic medicine *Rhus tox* 6C (prepared from *Toxicodendron radicans*) [Figure 1 and Table 1] on a newly prepared primary midgut cell culture of *A. albopictus*.

After a comprehensive review of literature, we noticed that propagation of DENV is best observed in mosquito midgut cells, which was originally observed by researchers in organ culture, as it is the primary target site of viral invasion.[8] After entry of DENV in mosquitoes, main pathological changes are found in the midgut and salivary gland cells.[2,9-11] Among them, it was presumed that study of midgut cells is the best tool which mimics the real biological interaction between host, vector, and pathogen.[12]

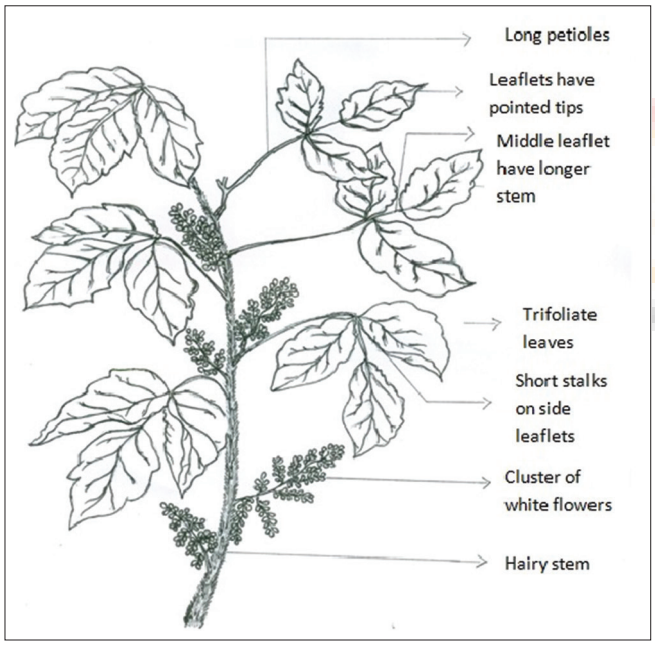

Figure 1: *Toxicodendron radicans* plant

Table 1: General characteristics of the medicine *Rhus toxicodendron*

Parameters	Features
Homoeopathic medicine	*Rhus toxicodendron*
Source plant	*Toxicodendron radicans*
Family	*Anacardiaceae*
Part used for preparing homoeopathic medicine	Leaves
Active compounds	Urushiol (mixture of phenolics compounds called pyrocatechol, i.e., 1, 2-hydroxybenzene)

Available mosquito cell lines are either prepared from larvae or eggs,[13] and these established cell lines are commonly used for propagation of DENV[14] and related flaviviruses although these cells do not represent the natural host-pathogen interaction, which could be observed in the midgut cells of mosquitoes.

Hence, with an aim to study the real biological interaction of DENV with the vector as well as the host cells, a new attempt has been made in this initial phase of our project, to expand cell lines from midgut of locally available wild *A. albopictus* mosquitoes in West Bengal. This new primary cell culture was standardized by us, and it is expected to facilitate studies involving interactions of viral antigens with specific receptors, microRNA, silencing genes, and other molecular markers; a standardized midgut cell culture will also assist in comparative studies of pathogenicity, as well as drug development toward many viral and parasitic diseases, in which mosquitoes are the only vectors. The standardized protocol of the preparation of *A. albopictus* midgut cell culture developed by us and the direct effect of a homoeopathic medicine *Rhus tox* 6C on these cells are presented here.

Materials and Methods

Collection and Sterilization of *Aedes albopictus* Mosquitoes

Mosquitoes were trapped from the surrounding areas of DACRRIs building, near Dakshineswar (22.6554° N, 88.3579° E) by mosquito nets. The collected mosquitoes were identified by Professor A.K. Hati, Ex-Director and Head, Department of Entomology, School of Tropical Medicine, Kolkata, based on classical descriptions.[15] The collected mosquitoes were anesthetized[16] by keeping them at low temperature (~4°C). The mosquitoes were washed with sodium dodecyl sulfate solution (10% [v/v]), followed by distilled water, sodium hypochlorite solution (0.1% [v/v]), and again distilled water for 2 min in each solution.[17]

Mosquito Dissection and Separation of Mosquito Midgut

The mosquito was then placed on a drop of phosphate buffer saline (PBS) on glass slide, followed by dissection and separation of the midgut. The separated midgut was then sterilized by immersing consecutively in insect physiological solution (IPS) with sodium hypochlorite solution, IPS without sodium hypochlorite solution, and distilled water as illustrated in Figure 2. After sterilizing the midgut, it was gently macerated to make homogeneous cell suspension in Dulbecco's Modified Eagle's Medium (DMEM) culture medium.[18]

Morphological Study of Midgut

The midgut was treated with 1 M NaOH solution, and after 30 min, the midgut was observed microscopically at ×100 magnification.

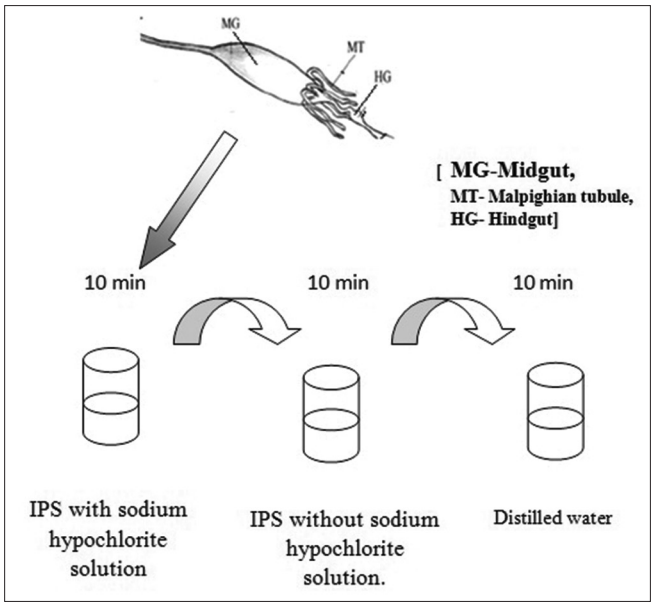

Figure 2: Sterilization procedure of midgut of *Aedes albopictus*

Histological Study of Midgut of *Aedes albopictus* Mosquitoes

Paraffin blocks of the midgut were prepared, sections were made in a microtome, and then the sections were stained with hematoxylin and eosin (H and E) staining method and mounted with a mixture of distyrene, plasticizer and xylene.

Aedes albopictus Midgut Cell Culture

After mosquito dissection and separation of midgut, the isolated midgut cells were inoculated in 6-well culture plates (1.2×10^6 cells/well) containing DMEM culture medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco), sodium bicarbonate (3.7 g/L) (Sigma, USA), penicillin-streptomycin (100 U/mL and 100 µg/mL) (Gibco), and antimycotic solution (amphotericin B-0.25 µg/mL) (Gibco). The culture plates were kept in incubation at 28°C till the cells became confluent (~72 h).

Subculturing of *Aedes albopictus* Midgut Cells

At around 75% confluency, the cells were counted in a hemocytometer chamber. Fresh DMEM culture medium (Gibco, USA) supplemented with 10% FBS (Gibco), sodium bicarbonate (3.7 g/L) (Sigma, USA), penicillin-streptomycin (100 U/mL and 100 µg/mL) (Gibco), and antimycotic solution (amphotericin B-0.25 µg/mL) (Gibco) was added to the wells. Then, the cells were aspirated gently and removed from the culture flask and subcultured in 1:2 ratio in DMEM culture medium supplemented with 10% FBS, sodium bicarbonate, penicillin-streptomycin and antimycotic solution and maintained at 28°C and 5% CO_2 for 72 h to reach confluency.

Preliminary Observation of Cytopathic Effect of Wild Dengue Virus on *Aedes albopictus* Primary Midgut Cell Culture

Serum samples collected aseptically from three patients suffering from dengue fever for 2–3 days with NS1 Ag levels more than 30 units, indicating high levels of viremia, were used to infect confluent *A. albopictus* midgut cell culture. The infected cell culture was incubated at 28°C and 5% CO_2 for 2–7 days or until cytopathic effect (CPE) appeared. Control studies were also made with three NS1 Ag negative serum samples.

Scanning Electron Microscopy Analysis of Cells of Midgut of *Aedes albopictus*

For electron microscopy, the sample was washed with PBS followed by fixation in glutaric dialdehyde and subsequently with osmic acid on glass slides of definite measurements. The cells were then subjected to increasing gradient of alcohol followed by incubation in a CO_2 chamber to dehydrate the sample. Finally, the glass slides containing sample were mounted on studs, and the samples were coated with gold in S150 sputter coater of Edwards. After gold coating, the samples were observed and analyzed in FEI QUANTA 200 (Bose Institute, Rajabazar, Kolkata, West Bengal, India) at high vacuum mode. The cells on the "test" (*Rhus tox* 6C treated) and the "control" (succussed alcohol treated 6C) slides were observed under scanning electron microscopy (SEM).

Cell viability assay by trypan blue exclusion method

Midgut cell cultures (1.5×10^5 cells/mL) were prepared in 96-well tissue culture plates containing DMEM culture medium and incubated at 28°C and 5% CO_2. The cell viability was determined before inoculation by mixing equal volume of 0.4% trypan blue solution (Sigma, USA) (1:1) to cell suspension. Viable and dead cells were counted under phase contrast microscope. The percentage of cell viability was calculated using the following formula:

% viability = (Live cell count/total cell count) × 100

Cell viability assay by cell counting kit 8 method

Midgut cell cultures were prepared, and cells were inoculated in serial dilutions (1.6×10^3 cells, 8×10^2, 4×10^2, 2×10^2, 1×10^2) in 96-well plates containing DMEM culture medium and incubated for 0, 12, and 48 h. After incubation, 10 µL of water soluble tetrazolium solution of cell counting kit 8 was added to each well and incubated for 3 h at 28°C and 5% CO_2. After incubation, the absorbance of each well was measured at 450 nm and was represented graphically against a number of cells.

Application of *Rhus tox* 6C on *Aedes albopictus* Primary Midgut Cell Culture

In this experiment, 100 µL of *Rhus tox* 6C was applied to the 6 well "test" cell culture plates and 100 µL of succussed alcohol 6C to 6 well "control" cell culture plates. Then, the cell culture plates were observed under inverted microscope at ×400 magnification.

Then, to study the morphological characteristics of the cells, the cells of both "test" and "control" groups were stained with Leishman's stain and the morphological changes were evaluated statistically.

Statistical Analysis

Under inverted microscope, different microscopic fields were selected at random. At ×400 magnification, the morphological characteristics of the cells treated with *Rhus tox* 6C ("test") were studied along with cells treated with succussed alcohol 6C ("control"). The parameters that were taken into consideration were total number of cells, number of round cells, number of deformed cells, number of diploform cells, number of chains, and number of clumps (≥3 cells). Based on the above parameters, statistical analysis of the effect of *Rhus tox* 6C on the cells of the "test" cell culture plates was done compared to the cells of the "control" cell culture plates.

Results

The morphological characteristics of *A. albopictus* midgut tissue when studied under inverted microscope at ×100 magnification after treatment with 1 M NaOH solution showed a network of branching tubules known as tracheoles on the surface of midgut [Figure 3a] and distinct arrangement of residential cells containing rectangular-shaped cells, which were identified as columnar epithelial cells, a small number of goblet cells, regenerative cells, and round stem cells [Figure 3b]. Histopathological analysis after H and E staining showed pseudostratified lining of cells, which was sometimes discontinuous. The smooth muscle cells and fibrous cells were also seen organized representing muscular and serous coats. The predominant mucous columnar cells were found in clumps.

After application of NS1 Ag positive serum containing wild DENV, typical DENV-induced CPE [Figure 4b] was observed within 48 h which was remarkably absent in all control experiments with NS1 Ag negative serum [Figure 4a]. The main changes were markedly swollen cells in big clumps [Figure 4b].

The electron microscopic study of primary culture of *A. albopictus* midgut cells showed that the cells of "test plate" (*Rhus tox* 6C treated cells) [Figure 5b] were large in size, more in number, and were more organized than the cells of "control plate" (succussed alcohol 6C treated cells) [Figure 5a].

The cell viability was determined by trypan blue assay and the % viability was found to be 80% [Table 2] at the time of cell inoculation in cell culture plates.

The rate of proliferation of primary midgut cells in culture over increasing incubation time is shown in Figure 6. The viability assay indicates a higher growth rate at 48 h in comparison to growth rate at 0 and 12 h.

After observations of all the four sets in this experiment, i.e., on observation of cells of the "test" cell culture well, compared to the "control" cell culture well under inverted microscope at ×400 magnification in all the sets, it was identified that the cells treated with *Rhus tox* 6C [Table 3 and Figure 7] were mostly single cells and few were in clumps compared to the cells of the "control" cell culture well, where most cells were in clumps. In addition to that, the structure of the cells of the "test" cell culture well was found almost intact and healthy, in contrast to the mostly deformed cells of the "control" cell culture well [Figure 8].

Discussion

Arboviruses, gametocytes of malaria parasites, and microfilaria enter the mosquito through an infectious blood meal, the

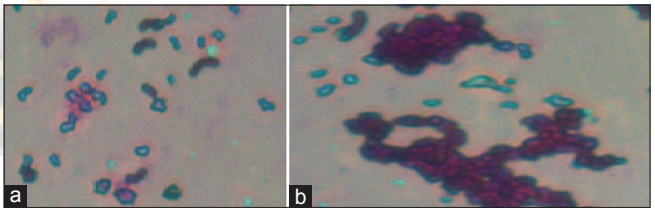

Figure 4: (a) Cytopathic effect of primary midgut cells after 48 h posttreatment with NS1 Ag negative serum. (b) Cytopathic effect of primary midgut cells after 48 h postinfection with wild dengue virus (NS1 Ag positive serum)

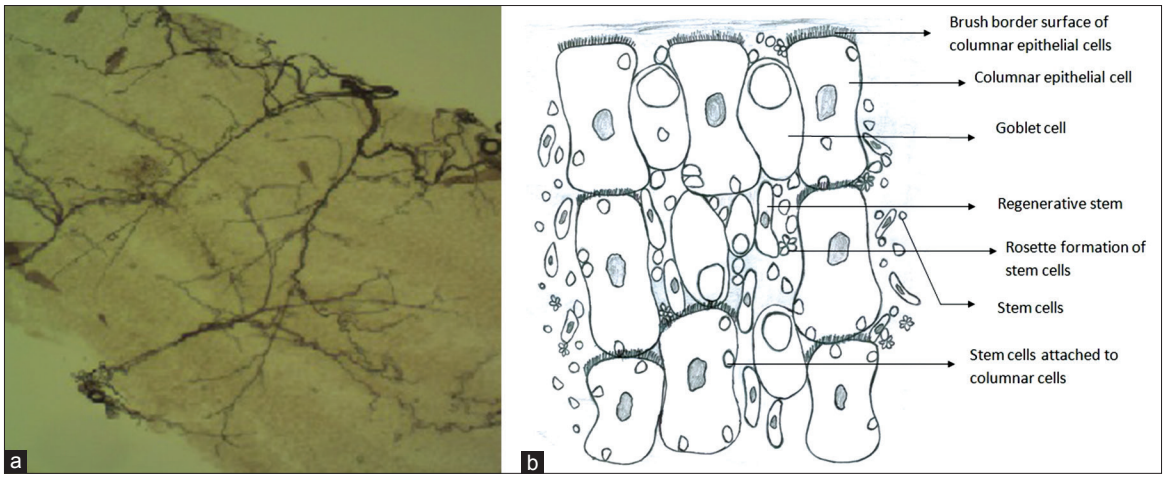

Figure 3: (a) Tracheoles were commonly found on the surface of midgut as branching tubules (×400). (b) Pseudostratified cellular morphology of different cells on midgut surface of *Aedes albopictus*

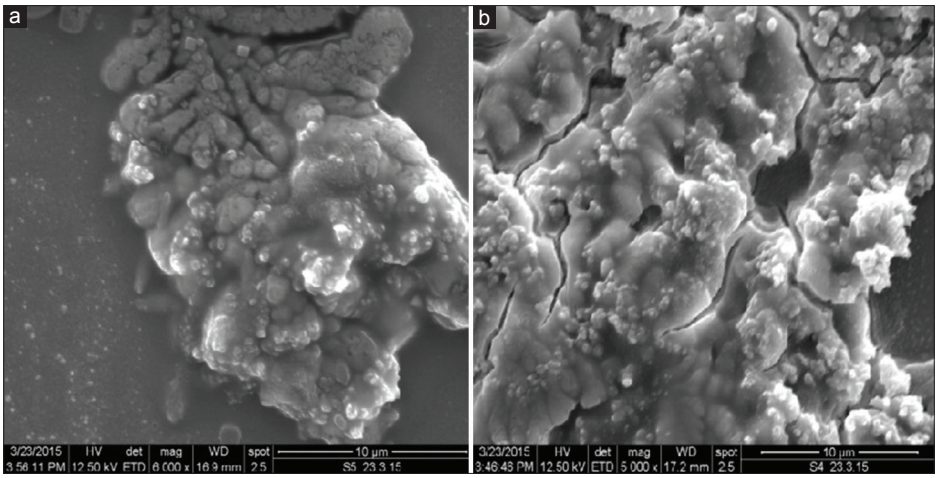

Figure 5: (a) Cells of "control" (succussed alcohol treated 6C) plate under scanning electron microscopy (×6000). (b) Cells of "test" (*Rhus tox* 6C treated) plate under scanning electron microscopy (×5000)

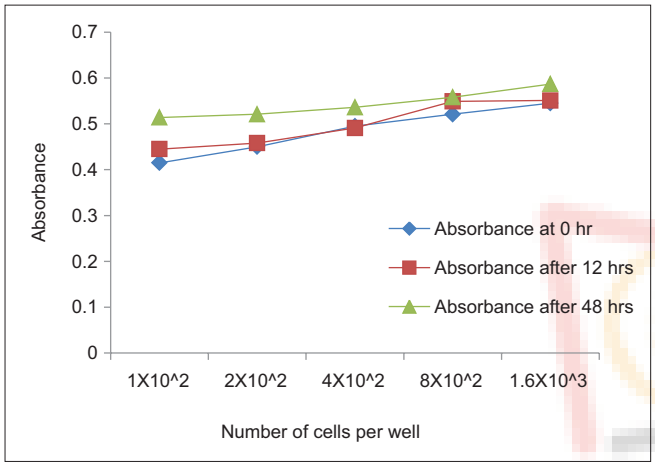

Figure 6: Viability assay of primary midgut cell culture with increasing time of incubation

Table 2: Percentage of cell viability

Viability (%)	Live cell count	Total cell count
80 (cells/mL)	1.56×10^3	1.95×10^3

Table 3: Statistical interpretation of *Rhus toxicodendron* 6C induced changes in cell culture derived from midgut of *Aedes albopictus*

Field	Control plate			Test plate		
	Mean	SD	SEM	Mean	SD	SEM
Total number of cells	220	84.86	28.3	131	40.9	13.64
Round cells	109	41.67	13.89	63	19.06	6.35
Deformed cells	43	20.15	6.72	33	19.07	6.36
Chain cells	0	0.7	0.23	1	2.12	0.71
Diploform cells	5	3.04	1.01	2	1	0.33
Clump cells	3	2.08	0.69	2	1.58	0.53

SEM: Standard error of mean; SD: Standard deviation

pathogens then cross the peritrophic membrane, pass through the membranous microvilli-associated network, make contact with microvillar surface, penetrate into the midgut pseudostratified epithelium, and finally disseminate and multiply in other parts of the mosquito. According to the abundance of microvilli, the cells of midgut are divided into two types - the predominant type is the columnar cells with dense microvilli, which are interspersed with the other variety of cells with fewer microvilli, found particularly in the posterior part of the midgut, where invasion of pathogens commonly occurs. These cells in the posterior part of the midgut are with plenty vesicular ATPase. The pseudostratified lining cells of the midgut are placed on the basement membrane, surrounded by muscle fibers, nerve fibers, and tracheoles. According to predominant function, the midgut cells are classified into secretory cells containing basal membrane labyrinth, common absorptive cells without basal membrane labyrinth, and smaller endocrine cells which are again divided into various types on the basis of the presence of secretory granules, electron density, and reactivity to specific antipeptide hormone antisera. Besides these cells, there are also goblet cells and small, basally located regenerative cells originating from stem cells. The arrangement of cells is illustrated in Figure 3. A semi-stable (usually all primary cultures are semi-stable as within months dividing cell population gradually decreases) balance between stem cell proliferation, differentiation to columnar and goblet cells, and death of these mature cells occurs in a primary midgut cell culture as observed by us.

A crucial point of microbial transmission is their attachments on receptors of midgut cells which are oligosaccharides on midgut glycoproteins.[19-21] A high proportion of N-linked GlcNAc- and GalNAc-terminal oligosaccharides are present in these glycoproteins.[22] Sugar epitopes may be used as targets to block transmission of infectious agents. A broad understanding of these sugar structures is essential for experiments based on these targets. Mosquito cell lines derived from larvae have already been used to study the lectin-mediated adhesion system.[23] In midgut of hemipteran

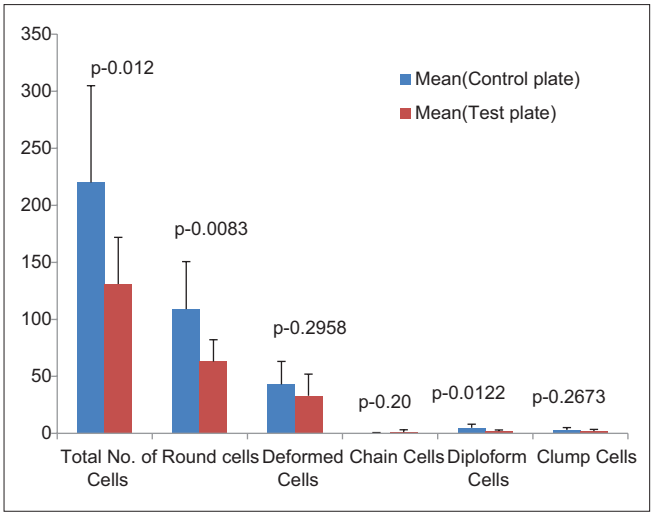

Figure 7: Statistical interpretation of *Rhus tox* 6C induced changes in cell culture derived from midgut of *Aedes albopictus*

insects, an unusual extracellular lipoprotein membrane is found covering the microvilli of midgut cells. This membrane is known as perimicrovillar membrane containing α-glucosidase, and the space between the perimicrovillar membrane and the microvillar membranes is known as the perimicrovillar space;[24] however, this space is not reported in mosquitoes.

Thus, a new mosquito midgut cell line developed by us may be an ideal *in vitro* model for the study of biological characteristics of DENV propagation in a natural way, and future study of possible interactions of different homoeopathic medicines at all pivotal molecular steps inside the cell.

Primary midgut cell cultures were semi-stable for about 1 month, with a slowly decreasing population of dividing cells. During this 30 days period, about 10% of existing columnar and goblet cells are dying and are replaced by differentiating round stem cells.

Conclusion

In this study, we observed that *Rhus tox* 6C increases cell growth and organization in the culture plate as observed under SEM, while the rate of deformity and clumping of cells have also decreased.

There is no study on direct action of *Rhus tox* 6C on any cell line till date, and only a few studies have been done on its biological activities. Thus, this study will open up a new avenue of future studies with this new primary cell culture, for the benefit of the humanity at large.

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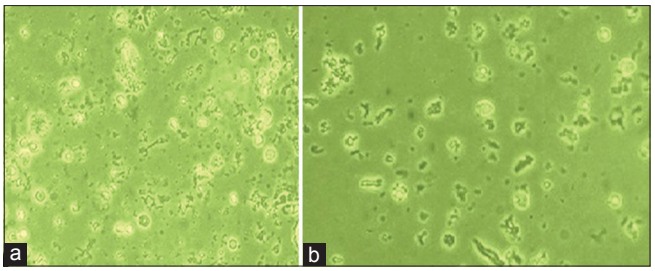

Figure 8: (a) Microscopic view (×400) of midgut cells of control plate (succussed alcohol 6C) showing many deformed and clumped cells. (b) Microscopic view (×400) of midgut cells of test plate (*Rhus tox* 6C treated) showing mostly normal cells

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Conflicts of Interest

There are no conflicts of interest.

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Beobachtung einer direkten Veränderung der Mitteldarmzellen von *Aedes albopictus* (Tigermücke) durch *Rhus toxicodendron* C6 in direktem Verhältnis zu Dengue-Virus Infektion

Zusammenfassung

Hintergrund und Ziele: Der Eintritt des Dengue-Virus in die Mücke als Krankheitsüberträger geschieht über die Mitteldarmzellen. Verfügbare Zelllinien von Mücken für In-vitro-Untersuchungen des Dengue Virus (DENV) werden aus Eiern oder Larven hergestellt, die keine geeigneten Modelle darstellen, die Infektiösität zu untersuchen. Daher entwickelten wir eine neue primäre Zellkultur der Mitteldarmzellen von Aedes albopictus. Diese wurden für die in vitro Untersuchung von DENV standardisiert, um einen möglichen Einfluss homöopathischer Arzneien zur Vorbeugung und Reduktion der DENV-Invasivität der Mitteldarmzellen zu zeigen. Diese primären Mitteldarmzellen demonstrierten eine herausragende zytopathische Wirkung bei der Infektion mit wild isolierten DENV von infizierten Dengue Patienten in der Virämiephase. **Materialen und Methoden:** In dieser Arbeit konnten wir einen direkten Einfluss des homöopathischen Mittels *Rhus toxicodendron* C6 (*Rhus* tox C6) (Verdünnung 10-12) auf die primären Zellkulturen beobachten, mit dem Ziel signifikante Veränderungen zu finden, die als Basisdaten bei weiteren Experimenten zum möglichen Einfluss von *Rhus tox* gegen die DENV-Infektion in diesen Zellen dienen. Folglich können diese direkten Veränderungen eine Voraussetzung für die Wirksamkeit dieser Arznei gegen das Eindringen von DENV sein; so wird ein üblicherweise verwendetes homöopathisches Arzneimittel gegen Dengue Fieber verwendet. **Fazit und Diskussion:** In unseren Versuchen fanden wir unter dem Elektronenmikroskop, dass durch *Rhus tox* C6 die Größe der Zellen zunehmen und bei der Organisation der Zellen auf festem Untergrund behilflich sein konnte, obwohl die Anzahl der Zellen zurückging. Zudem waren mit Rhus tox C6 behandelte Zellen gesünder, erkennbar an der geringeren Anzahl deformierter, geklumpter und diploformer Zellen.

Observación sobre los cambios directos que ejerce *Rhus tox* 6c en las células del intestino medio de *Aedes albopictus* en relación con la infección por el virus del Dengue

RESUMEN

Fundamento y Objetivos: En los mosquitos vectores, la invasión por el virus del dengue (dengue virus, DENV) se produce a través de las células del intestino medio. Sin embargo, las líneas celulares disponibles del mosquito para el estudio *in vitro* del DENV se preparan a partir de huevos o larvas, los cuales constituyen modelos inapropiados para examinar la infecciosidad. Por ello, hemos desarrollado un nuevo cultivo celular primario a partir del intestino medio del mosquito *Aedes albopictus*. Dicho cultivo ha sido estandarizado para el estudio *in vitro* del DENV, con el objetivo de encontrar cualquier posible efecto de los medicamentos homeopáticos para prevenir o reducir la invasividad del DENV en estas células del intestino medio. El cultivo celular primario del intestino medio mostró efectos citopáticos prominentes en la infección por DENV salvaje cultivado a partir de pacientes con infecciones por dengue en la fase virémica.

Materiales y métodos: En este artículo, hemos observado el efecto directo del medicamento homeopático *Rhus toxicodendron* 6c (*Rhus tox* 6c) (ultradilución de 10−12) sobre este cultivo celular primario, para examinar los cambios significativos. Se pretende utilizar estos cambios como datos básicos para los futuros experimentos sobre el posible efecto de *Rhus tox* 6c frente a las infecciones por DENV en estas células. Por ello, estos cambios directos pueden ser un requisito para la acción de este medicamento contra la invasión por DENV, ya que se trata de uno de los medicamentos homeopáticos comunes del repertorio utilizados en la fiebre del dengue. En nuestros experimentos, hemos observado que *Rhus tox* 6c puede aumentar el tamaño de las células y contribuir

Conclusiones y discusión: A la organización de las células sobre la superficie sólida, tal y como se ha observado bajo microscopio electrónico de barrido, pese a que se produjera una reducción del número total de células. Además, las células tratadas con *Rhus tox* 6c eran más sanas, ya que se constató un menor número de células deformadas, aglomeradas o diploformes.

डेंगू वायरस संक्रमण संबंधी रसटॉक्स 6सी द्वारा एडिस एलबोपिक्टस आद्यमध्यांत्र कोशिकाओं के प्रत्यक्ष परिवर्तन पर एक अवलोकन

सार

पृष्ठभूमि और उद्देश्यः मच्छर वैक्टर्स में, डेंगू वायरस (डीईएनवी) का आक्रमण आद्यमध्यांत्र कोशिकाओं के माध्यम से होता है लेकिन डीईएनवीके इन–विट्रो–कृत्रिम परिवेशीय अध्ययन के लिए उपलब्ध मच्छर कोशिका, अंडे या लार्वा से तैयार होती हैं, जो इसके संक्रामक अध्ययन के लिए उपयुक्त मॉडल नहीं है। इसलिए, हमने एडिस एलबोपिक्टस मच्छर आद्यमध्यांत्र से एक नया प्राथमिक कोशिका जीवाणु विकसित किया और डीईएनवीके इन–विट्रो–कृत्रिम अध्ययन के लिए मानकीकृत किया, इस उद्देश्य के साथ कि इन आद्यमध्यांत्र कोशिकाओं में रोकने या डीईएनवी इनवैसिवनेस सुध ाारने में होम्योपैथी औषधियों की किसी भी संभावित भूमिका का पता लगाया जा सके। इस आद्यमध्यांत्र प्राथमिक कोशिका जीवाणुओं ने वरमिक चरण में डेंगू से संक्रमित रोगियों से अलग उग्र डीईएनवी के साथ संक्रमण पर प्रमुख कोशिका विकृति संबंधी प्रभाव प्रदर्शित किया।

सामग्री और पद्धतिः महत्वपूर्ण परिवर्तनों का पता लगाने के लिए, इस लेख में हमने इस प्राथमिक कोशिका जीवाणुओं पर होम्योपैथी दवा रस टॉक्सिकोडेंड्रोन 6सी (रस टोक्स 6सी) (10–12का अल्ट्रा डाइल्युट) का प्रत्यक्ष प्रभाव पाया, इन कोशिकाओं में डीईएनवी संक्रमण के खिलाफ रस टॉक्स 6सी की संभावित भूमिका का पता लगाने के लिए इसे भावी प्रयोगों में आधारभूत डेटा के रूप मे इस्तेमाल किया जाएगा। इसलिए, यह प्रत्यक्ष परिवर्तन डीईएनवी आक्रमण के विरुद्ध इस दवा की क्रियाशीलता के लिए एक आधार स्वरूप हो सकता है। क्योंकि डेंगू बुखार में इस्तेमाल की जाने वाली आम होम्योपैथिक दवाओं में से यह एक है।

निष्कर्षः हमारे प्रयोगों में हमने पाया है कि रस टॉक्स 6सी कोशिकाओं के आकार में वृद्धि करने में सक्षम है और जैसा कि स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप पर देखा गया यह ठोस सतह पर कोशिकाओं के गठन में मदद कर सकता है, हालांकि कोशिकाओं की कुल संख्या में कमी पायी गयी। इसके अलावा, रस टॉक्स 6सी द्वारा उपचारित कोशिकाएं अपेक्षाकृत स्वस्थ थी जो कम संख्यक विकृत में झुंड, और डिप्लोफार्म कोशिकाओं द्वारा स्पष्ट होता है।