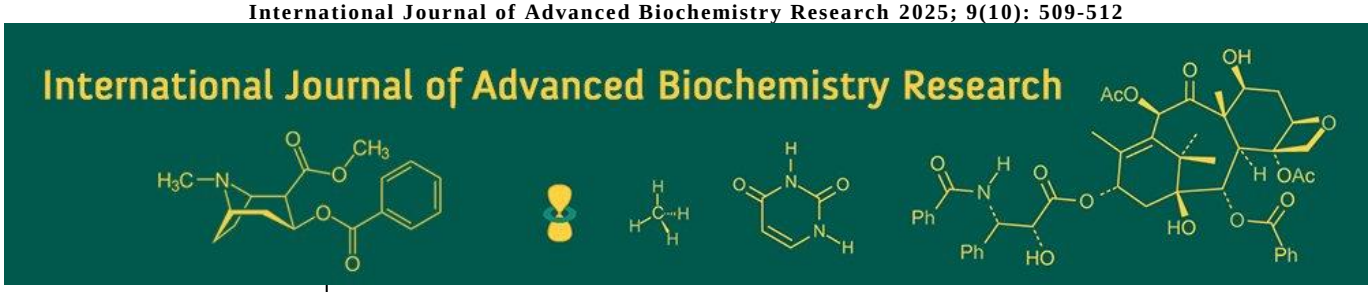

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A noval explanation for Traveller's diarrhoea and traveller's headache

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Abstract
Background: Travellers Diarrhoea is a common disease among young adults travelling from cold or temperate countries to hot tropical countries characterised by loose stools 2-3 times per day often resolve by its own within 2 days. Common aetiology is found to be enterotoxigenic E coli bacteria, stress etc.
Objective: To evaluate the effect of temperature on activity of ALT, LDH to extrapolate effects of temperature on human enzyme activity.
Methods: Serum from left over samples of patients with normal and abnormal levels of ALT and LDH were separated stored at 8, 37 and 50 degree Celsius respectively for 4 hours. The test is performed after 4 hours in automated biochemical analyzer Mindray BS600 and the results were recorded. Results were plotted against the temperature in graph and studied.
Results: From the graph we found that in the sample store at 8 degree Celsius, the activity of LDH and ALT increased consistently with concentration of enzymes in the blood. But in samples stored at 37 and 50 degree Celsius there were some variations. Also activity of LDH incresesd till 37 degree Celsius and decreased till 50 degree Celsius whereas activity of ALT gradually decreased with increasing temperature.
Conclusion: Traveller's diarrhoea is caused due to sudden change in temperature which results in abrupt increase in enzyme activity in intestinal cells stimulating release of ions and water into the gut. Travellers disease is a personalised disease that depends on an individuals enzyme adaptability, concentration in the blood and stability. Young adults are often affected as thermal adaptability of enzymes may not be achieved in young and first time travellers. Traveller's headache is caused due to decreased emptying of sinusoidal secretion due to sudden decrease in temperature. Change in temperature and climate also trigger enterogenic microbes to increase or decrease in activity causing infections. So the travellers are advised to get acclimatised to the climate or conditions of the destination country before travelling to those countries. But the current study lacks quantitative data of the intestinal enzymes to explain the phenomena. Also the study need to be extended with quantitative enzymatic study in both climatic conditions and also bacteriological study of gut microbiota in Travellers from both the climatic conditions which requires more funding.
Conflicts of Interest: Author certify that there is no involvement of any third party funding or interest in this Research.

Keywords: Traveller' diarrhoea, Traveller's headache, enzyme equilibrium, acclimatisation

Introduction
Traveller's diarrhoea is considered as a syndrome. Diarrhoea is caused due to release of ions and fluid into the intestinal lumen from the intestinal cells. It is supposedly caused by the activity of entero-toxigenic *E. coli* in traveller's diarrhoea. Toxins released from E coli act on the gut epithelium and triggers enzymatic process to release ions and water into gut lumen [1]. Dysentery, on the other hand is caused due to damage of epithelial cells on the intestinal tract often caused by bacteria like Salmonella Typhi and Entamoeba histolytica. Other causes of diarrhoea include various viruses, protozoans and many bacteria. Antibiotic prophylaxis and anti-diarrhoeal or anti-motility drugs like loperamide, Bismuth and atropine are the drugs of choice [2]. As the people travelling across countries rises, we need to develop methodologies and therapeutics to diagnose this early, prevent the incidence or treat it effectively. Travellers also complaint of change of plans due to this unpleasant condition. Traveller's diarrhoea is often observed in traveller's from cold temperate countries moving to hot and tropical countries.

Clinically it is presented as mild ailment to severe case which requires hospitalisation. Irritable bowel syndrome is another consequence related to it [3]. Dietary avoidance measure is not found to be useful in preventing travellers diarrhoea [4]. So international traveller's are often advised to carry required preventive drugs. It is often associated with abdominal cramps making it unpleasant. It affects 30% to 70% of the travellers affecting across different seasons and destinations. People travelling to most part of Asia, Middle East, Africa, Mexico, Central and South America are found to be at risk of developing the disease. Mild symptoms starts within 6 hours to 24 hours and persists for 2-3 days [5]. Young adults are often found to develop traveller's diarrhoea than old travellers. Oregano oil and Echinacea extract are also proven to be used for therapy [6].

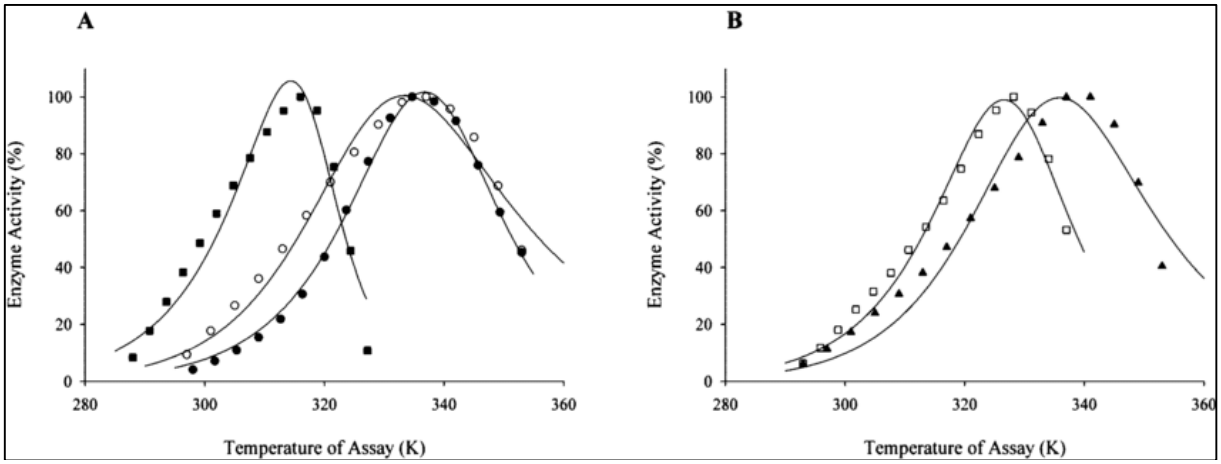
One of important variable that affect an enzyme activity is temperature. In a biological system temperature affects numerous processes including enzyme catalysis, protein stability and temperature dependent regulation [7]. Arrhenius (k = Ae(−Ea/RT) and Eyring equations (k = (kB*T/h)e^(dG^++/(kB*T)) explains the temperature dependence of a chemical reaction including enzymatic reactions. The Boltzmann constant, denoted as kB, is a fundamental physical constant with a value of approximately 1.38×10^-23.It relate a system's temperature to average kinetic energy of it molecules. It can be applied in this scenario to explain the relationship between amount of enzyme present in the body and its potential to increase the activity with increase in temperature.

But the enzymes does not strictly follow these equations. The Equilibrium model explains the thermal reactions of enzymes which states the presence of an active and inactive forms of the enzymes and active form of the enzyme is only present at a narrow range of temperature because of the thermal variation of geometry of active site of the enzyme [8]. In this model, the active form of the enzyme (Eact) is in reversible equilibrium with an inactive (but not denatured) form (Einact), and it is the inactive form that undergoes irreversible thermal inactivation to the thermally denatured state (X) [8].

$$E_{act} \leftrightarrows E_{inact} \rightarrow X$$

Arrhenius activation and thermal stability are the two parameters that explains association of temperature with enzyme activity. The dependence of enzyme activity on temperature can also be explained by T(eq) a third thermal parameter. T(eq) is central to physiological adaptation of an enzyme to its environmental temperature and links the molecular, physiological and environmental aspects of adaptation of life to temperature.

Figure:1 [12]



The effect of temperature on enzyme activity has also been recently explained by MMRT (Macromolecular rate theory) theory derived from thermodynamics and heat capacity of enzymes [7].

Methods and Materials

Study populations

The study is conducted at the Biochemistry Lab of New Civil Hospital, Surat, located in South Gujarat of India. Left over serum samples of patient is collected for the study.

Ten samples with elevated, normal and abnormal low levels of common enzymes like ALT and LDH is selected for the experiment. The samples are separated using a 3000 rpm centrifuge to separate serum from the blood properly, which is further pipetted into three Eppendorf cups, 200 microliter each. First cup is placed at 8'C for 4 hours, second cup is placed at 37'C for 4 hours and third cup is place at 50'C for 4 hours.

The sample is evaluated for ALT and LDH after 4 hours in automated analyzer, MINDRAY BS600.

Statistical analysis

Linear regression analysis of Temperature vs enzyme activity is done. The graph is plotted to determine the association of temperature with enzyme activity.

Results and Discussion

Baseline characteristics

Table 1: Clinical and laboratory characteristics of the study

Parameter	Normal Range	Unit
ALT	<45	U/L
LDH	<360	U/L

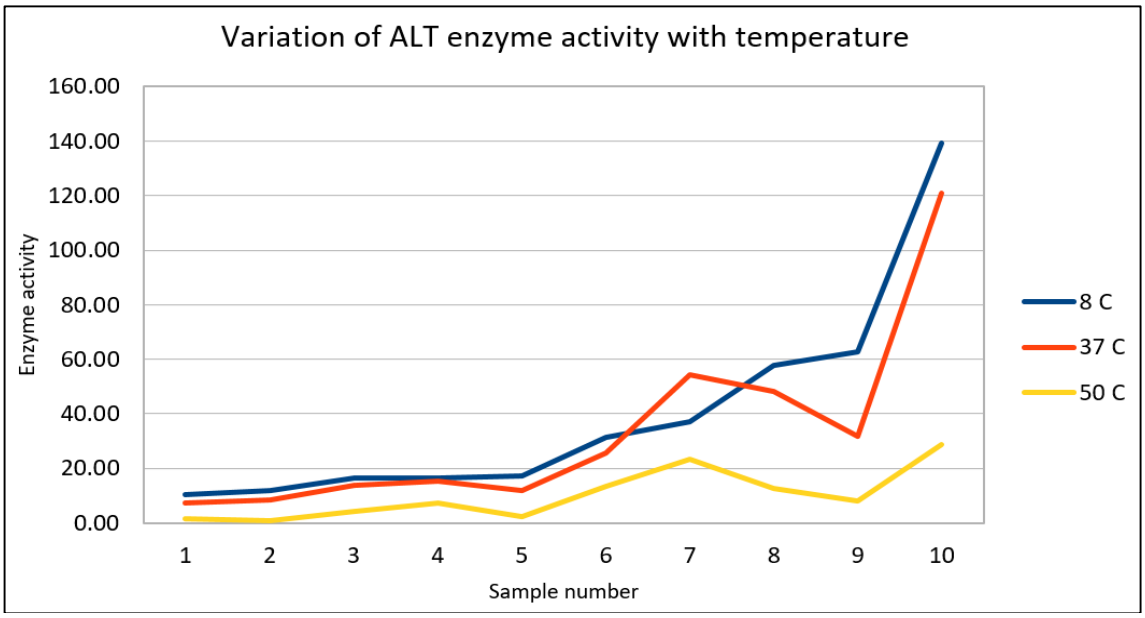
Abbreviations: ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, U/L: Unit/Litre.

Table 2: Average activity of enzyme at specific temperature

Parameter	8'C	37'C	50'C
ALT	40	33.7	10.2
LDH	246.23	252.16	180.86

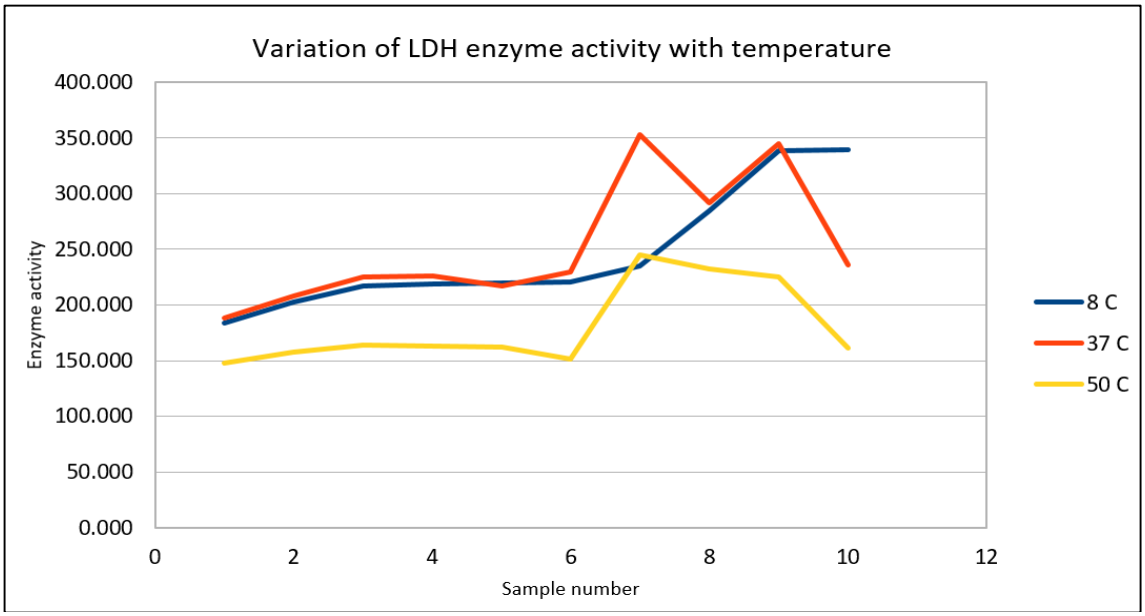
Abbreviations: ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, 'C-degree Celsius

Decreased activity of ALT at 37'C compared to 8'C is explained by the concentration/rate theory or by Boltzmann factor. So if the concentration of enzyme is less in the sample, the chance of getting denatured is very high. Although every enzyme has different optimal temperature at which it shows peak activity. So it is not possible to standardise a particular temperature as the ideal temperature for human enzyme activity.



Abbreviations: ALT: Alanine Transaminase

Graph 1: Variation of ALT enzyme activity with temperature



Abbreviations: LDH: Lactate Dehydrogenase

Graph 2: Variation of LDH enzyme activity with temperature

Graph shows smooth increase in enzyme activity-in both ALT and LDH in sample stored at 8'C but when we analyse samples stored at 37'C, we find that the increase in enzymatic activity is not linear or continuous because in this experiment we used 10 samples from 10 different individuals and the enzyme from each individual may have different temperature of equilibrium and so a sudden increase in temperature results in sudden decrease (due to destabilisation) or sudden increase(Arrhenius activation) in activity.

From the graph, it was interpreted that the average activity of ALT is maximum at temperature less than 37'C and its activity is less than its optimal activity at 37'C. The activity decreases with increasing temperature above 37'C. The activity of LDH increases with temperature till 37'C and decreases with further increase of temperature.

Activity of enzymes is directly proportional to amount of enzyme present in the sample. This is explained by Boltzmann factor, that is, the activity of enzyme is directly proportional to the kinetic energy acquired by the enzyme molecules. At low temperature the molecules does not acquire enough energy to carry out the reaction at the optimal speed. When the temperature is increased the enzyme molecules acquire more energy so that some of its molecules attain the optimal temperature and configuration for optimal enzyme activity. When the temperature is

further increased, the configuration of enzyme changes causing the loss of active site for the reaction to occur. The sudden decreases or increase in enzyme activity at change of temperature is caused due to adaptation of enzymes at a particular temperature for a while. This explains the sudden change in enzyme activity in the intestinal cells, and stimulation of release of ions and water into the intestinal lumen in people travelling from cold countries to hot countries that culminates in intestinal muscle cramps and diarrhoea creating unpleasant experience for the travellers. Young adults are often affected due to low acquired adaptability of the enzymes. This also explains the Traveller's headache, often observed in people travelling from hot and tropical countries to cold and temperate countries. Traveller's headache may be caused due to decreased movement of cilia and decreased emptying of secretions in sinuses often frontal sinus, resulting in deep dull aching headache. This theory also proposes possibility of traveller's constipation in people travelling from hot countries to cold countries.

Sudden change in temperature can also trigger some gut microbes to increase or decrease in activity to cause intestinal infections.

Conclusions

Traveller's diarrhoea is caused due to sudden change in temperature which results in abrupt increase in enzyme activity in intestinal cells stimulating release of ions and water into the gut. Travellers disease is a personalised disease that depends on an individuals enzyme adaptability, concentration of enzyme in the blood and stability of the enzyme. Young adults are often affected as thermal adaptability of enzymes may not be achieved in young and first time travellers. Traveller's headache is caused due to decreased emptying of sinusoidal secretion due to sudden decrease in temperature. Change in temperature and climate also trigger enterogenic microbes to increase or decrease in activity causing infections.

So the travellers are advised to get acclimatised to the climate or conditions of the destination country before travelling to those countries.

But the current study lacks quantitative data of the intestinal enzymes to explain the phenomena. Also the study need to be extended with quantitative enzymatic study in both climatic conditions and also bacteriological study of gut microbiota in Travellers from both the climatic conditions which requires more funding.

Abbreviations

E. coli: *Eschercia coli*
ALT: Alanine Transaminase
LDH: Lactate Dehydrogenase
rpm: Rotations per minute
'C: Degree Celsius
Eq: equilibrium
MMRT: Macromolecular rate theory

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All the authors contributed equally in the research. The author declare that there is no third part funding or interest in the study. The study is conducted in New Civil Hospital Biochemistry Lab, located at Surat, South Gujarat, India. Left over samples were used for the study from the laboratory.

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