APPENDICES

APPENDIX - 1

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam
Institute for Home Science and Higher Education for Women
University
(Estd. u/s 3 of UGC Act 1956)

Chairman
Dr. S. Ramalingam
Principal, PSG Institute
of Medical Sciences
& Research, Coimbatore

Member Secretary
Dr. P. R. Padma
Professor, Department of
Biochemistry, Biotechnology and
Bioinformatics

Members
Dr. P. Santhanakrishnan
Mr. K.Arulmoli (Legal Expert)
Dr. S. Prentakumari
Dr. A. Saraswathy
Mrs. S. Radha Devi
Dr. N.S. Rohini
Mrs. Judith Justin
Dr. S. Kowsalya
Dr.Subhashini K. Sripathi

14th March 2016

To
Ms. Thongam Chanu Anel
Department of Food Science & Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Madam,

Ref : Your presentation of the proposal No. AUW.IHEC.2013:41 entitled "Effect of supplementation of Hibiscus sabdariffa Linn on nutritional status and work performance of athletes engaged in traditional sports of Manipur" on 11th February 2014

In continuation with the submission of the necessary documents by you, the Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. AUW.IHEC.2013:41 entitled "Effect of supplementation of Hibiscus sabdariffa Linn on nutritional status and work performance of athletes engaged in traditional sports of Manipur" submitted by you. The Approval number for the same is AUW/IHEC-13-14/FSN-41.

We wish you all the best in your research endeavours.

Regards,
14/3/16
Dr.P.R.Padma
Member Secretary

Institutional Human Ethics Committee

INSTITUTIONAL HUMAN ETHICS COMMITTEE
MANIPUR UNIVERSITY

DECISION FORM

Ref. No. Ac/IHEC/MU/201/2014 Thursday, July 10, 2014

To

Dr. T. Inaobi Singh
Department of Physical Education and Sports Sciences
Manipur University
Canchipur 795 003

A meeting of the Institutional Human Ethics Committee (IHEC) of Manipur University was held on 9th July 2014 at 1:00 P.M at the Department of Biotechnology, Manipur University.

The following research proposal submitted on 17th Jan 2014 with relevant documents was discussed:

"Effect of Supplementation of Hibiscus sabdariffa linn on Nutritional Status and Work Performance of Thang Ta athletes of Manipur"

After detailed deliberation and review, the following decision was taken *(tick only one box, leaving others blank)*

- [x] Approved
- [ ] Disapproved
- [ ] Resubmit after modification
- [ ] Approved earlier but approval withdrawn now for the reason(s) detailed below

Dr. Lisam Shanjukumar Singh
Member Secretary

Member Secretary
Institutional Human Ethics Committee
Manipur University

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ꯍꯨ꯬ ꯂꯂꯣꯡ ꯃꯅꯤꯄꯨꯔ ꯊꯣꯡ-ꯇꯥ ꯀꯂꯥꯏꯖꯤꯡ

HUYEN LALLONG MANIPUR THANG-TA CULTURAL ASSOCIATION

Regd. No. 696 of 1966

Irilbung - 795 008, Imphal East, Manipur (India)

Ref. No. H/L-C-2014.

Date 5-02-2014

HUYEN LALLONG MANIPUR THANG-TA CULTURAL ASSOCIATION Regd. No. 696 of 1966 IRILBUNG

TO WHOM IT MAY CONCERN

This is certify that Kumari Thongam Chanu Anel of Singjamei Chingamakha Ningthoujam Leikai Imphal West is known to us Since 3 years She is Research Scholar of Department of Food Science and nutrition Avinashilingam Institute of Home Science and Higher Education for women Coimbatore. Regarding her Research Work her own Request to collect requisite data from our athletes for her research work on "In Title" Effect of Supplementation of Hibiscus Sabdariffa Linn on Nutritional Status and Work performance of athletes engaged on Indegenous Sports of Manipur, we agree.

She is not Related to me.

G. Gourakishor Sharma

G. Gourakishor Sharma

Director
Huyen Lallong Manipur Thang-Ta
Cultural Assn. Irilbung, Manipur (India)

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REGIONAL INSTITUTE OF MEDICAL SCIENCES
LAMPHELPAT, IMPHAL-795004, MANIPUR (INDIA)

Ref. No. 8/A/DPMR/09 Date 7th Mar, '15

To

Dr. P.R. Padma,
Member Secretary,
Institutional Human Ethics Committee,
Professor, Deptt. of Biochemistry,
Biotechnology & Bioinformatics,
Avinashilingam Institute for Home Science and Higher Education for Women,
Coimbatore – 641 043.

Sir,

This is with reference to your letter to Ms. Thongam Chanu Anel, dt. 21st February 2014.

That, I am giving my consent to be a member in the study panel on her study in **"Effect of supplementation of Hibiscus sabdariffa Linn on nutritional status and work performance of athletes engaged in traditional sports of Manipur"**.

With regards.

Yours faithfully,

(Dr. Y. Nandabir Singh)
Professor
Deptt. of Physical Medical & Rehabilitation
Regional Institute of Medical Sciences
Imphal, Manipur

Copy to:-
Ms. Thongam Chanu Anel,
Deptt. of Food Science& Nutrition,
Avinashilingam Institute for Home Science and
Higher Education for Women.

APPENDIX II

INFORMED CONSENT FORM

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings – including adverse events, if any, - whether directly or indirectly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements)

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date

Witness:

APPENDIX III

QUESTIONAIRRE
PART A

SOCIO-ECONOMIC STATUS OF THANG –TA ATHLETES IN MANIPUR

1. Name :
2. Age :
3. Sex : Male ☐ Female ☐
4. Religion : Hindu ☐ Muslim ☐ Christian ☐ Other ☐
5. Area of residence : Urban ☐ Rural ☐
6. Address :
7. Type of family: Joint ☐ Nuclear ☐ Extended nuclear ☐
8. Family Details:

S.No.	Name of the Members	Relation to the head of the family	Marital status	Education					Occupation	Income per month
				1	2	3	4	5		

1. Illiterate 2. Dropout 3. High School 4. Higher Secondary 5 Graduate 6 Post graduate

PART B

DIETARY ASSESSMENT

1. Are you a:

Vegetarian □ Non-vegetarian □ Ovo- vegetarian □

2. Which beverage do you drink in the morning?

Tea /coffee □Milk □Fruit juice □Water□

3. How many meals do you consumed per day?

2 times □ 3 times □ 4 timesuby

4. What are the types of fluids you consume?

a. Water □ b. Water and Juice □

Quantity (in l)

a. 1 to 2 □ b. 2 to 3 □ c. 3 to 4 □

5. Your diet:

a) □ is different every day b) □ is different only sometimes during a week

c) □ is different only during the weekend days d) □ is very monotonous

6. Do you have any known food allergies?

Yes ☐ No ☐

If yes, mention the food sources:

7. What types of food do you consume before competition?

a. Solid ☐ b. Semi solid ☐ c. Liquid ☐

8. How do you manage stress?

Music ☐ Books ☐ Yoga ☐ Sports ☐ Others ☐

9.Food frequency

Food Item	Daily	Weekly	Twice/ week	Thrice / week	Fortnightly	Monthly	Occasionally	Never
Cereals								
Rice								
Wheat								
Maize								
Pulses								
Peas								
Black gram								
Green gram								
Red gram								
Soybean								
Rajmah								
	Vegetables							
Amaranthus								
Cauliflower leaves								
Broad beans leaves								
Pumpkin leaves								

Food Item	Daily	Weekly	Twice/ week	Thrice / week	Fortnightly	Monthly	Occasionally	Never
Colocasia leaves								
Cabbage								
Coriander leaves								
Spinach leaves								
Cow pea tendril								
Knol-khol greens								
Mustard leaves								
Potato	**Other Vegetables**							
Onion								
Snake gourd								
Bottle gourd								
Cauliflower								
Plantain								
Cluster beans								
Ladies finger								
Papaya green								
Bamboo shoot								
Garlic	**Condiments and Spices**							
Ginger								
Turmeric								
	Fruits							

Food Item	Daily	Weekly	Twice/ week	Thrice / week	Fortnightly	Monthly	Occasionally	Never
Amla								
Banana								
Orange								
Guava								
Tomato								
	Meat,Fish and Poultry							
Fish								
Chicken								
Snail								
Pork								
Egg								
Milk and Milk products								
Milk								
Paneer								
Fats and oils								
Mustard oil								
Sugar and Jaggery								
Sugar								

10. Food frequency of Indigenous food

Food Item/ Scientific Name	Frequency							
	Daily	Once /week	Twice /week	Thrice /week	Fort nightly	Monthly	Occasionally	Never
Herbs								
Maroinapakpi/ *Allium hookerii*								
Maroinakupi / *Allium odorosum*								
Hangamyela/ *Brassica juncea* (L.) Czern								
Tokningkhok/*Houttuyniacordata*Thunb.								
Phakpai/*Persicaria capitata*								

Food Item/ Scientific Name	Frequency							
	Daily	Once /week	Twice /week	Thrice /week	Fort nightly	Monthly	Occasi onally	Never
(Buch.- Ham. ex D.Don) H.Gross.								
Yellang /*Polygonumorientale* Linn.								
Yaipal /*Curcuma angustifolia* Roxb.								
Namara /*Amomumaromaticu m* Roxb.								
Yaipal / *Curcuma angustifolia* Roxb.								
Yendem/*Alocasiaindi ca* (Roxb.) Schott.								
U- Hawaimaton / *Crotalaria juncea* Linn.								
Mayang ton /*Ocimum canum* Sims								
Pheija / *Wendlandia tinctoria* (Roxb.)DC								
Nongmangkhamapal /*Phlagocanthes thysiformis* Nees								
Sougri/ *Hibiscus*								
Shillo-Sougri/ *Hibiscus sabdariffa*								
Kolamni / *Ipomoea aquatica*Forsk.								
Komprek /*Oenanthejavanica* (Blume) DC.								
Heikakyelli / *Trapanatans*Linn.								
Tharo / *Nymphaea pubescence* Willd.								
Thambou / *Nelumbonucifera*(Ga ertn.)								
Thangjing / *Euryale ferox*Salisb.								
EshingKambong /*Zizanialatifolia*Turcz.ex								

Food Item/ Scientific Name	Frequency							
	Daily	Once /week	Twice /week	Thrice /week	Fort nightly	Monthly	Occasionally	Never
Loklei /*Hedychiumcoronarium*Koening.								
Pullei /*Alpinianigra*(Gaertn.) Burtt.)								
Mukthrubi /*Zanthoxylumacanthopodium* (Flower and leaves)								
Laphu / *Musa paradisiacal* Linn.(Stem)								
Yongchak /*ParkiaTimoriana*(A. DC.) Merr.Syn,P.roxburghiiG.Don (Beans and pods)								
Yendang /*Cycaspectinata*Hanilton. (Tendrils)								
Uchina (Black Slimy Mushroom or Wood Ear Mushroom)/*Auriculariadelicate* (Fr.)P. Henn								
Uyen /*Pleurotusostreatus*(Jacquin ex. Fr) Kummer.								
Chengum/ *Agaricuscampestris*								
Kanglayen (fungus)/*Schizophyllum commune*								
Fermented fish (ngari)/*Puntiussophore*								
Fermented soyabean (Hawaijar)/ *Glycine max* (L.) Merr.								

Food Item/ Scientific Name	Frequency							
	Daily	Once /week	Twice /week	Thrice /week	Fort nightly	Monthly	Occasionally	Never
Fermented bamboo shoot (Soibum) /*Bambusa*sp.								

11. Information about daily routine

a) Do you practice your game every day?
If yes, how many hours do you practice it?

i) 1h–2h in a day ☐ ii 3h–4h in a day ☐ iii) More than 4h in a day ☐

iv) >1 hour

12. 24 hour dietary recall method

Daily meals pattern

Meals	Menu	Amount	Ingredients
Breakfast			
Mid morning			
Lunch			
Mid afternoon			
Evening			
Dinner			
Bed time			

PART C

HEALTH PROFILE

Family history		Past History	
Disease	**Yes /No**	**Disease**	**Yes/No**
Diabetes		Anaemia	
Hypertension		Jaundice	

Heart disease		Malaria	
Asthma		Tuberculosis	
Tuberculosis		Athrititis	
Mental illness		Exercise induced Dizziness	
Cancer		Heat stoke	
Obesity		Head injury	
Sudden death<age 50		Fracture	
Personal history		Surgery	
Diabetes		Blood transfusion	
Hypertension		Chest pain – during exercise	
Heart disease		Racing of heart beat during exercise	
Hypoglycemia		Hospitalization	
Epilepsy		**Immunization**	
Allergies		Tetanus	
Asthma		Typhoid	
Sleeping disorders		Cholera	
Celiac disease			
Deverticulitus			
Crohn /IBD			

Disease from the past few weeks	Always	Often	Sometimes	Never	Hepatitis B	
Headache					Hepatitis A	
Diarrhoea					Measles	
Constipation					H. Influenza B	
Fever /cold					Chicken pox	
Indigestion					Yellow fever	
Personal habits						
Smoking						
Alcohol						
Food supplement						
Inhaler						
Vitamins						
Medications						

PART D

ANTHROPOMETRY

Name :

Address:

Phone No. :

Anthropometry	**Ist**	**2nd**	**3rd**
Age			
Height (cm)			

Weight (kg)			
Chest circumference (cm)			
Waist (cm)			
Abdomen Hip (cm)			
Upper arm relaxed (cm)			
Upper arm flexed (cm)			
Fore arm girth (cm)			
Thigh girth (cm)			
Calf girth (cm)			
Wrist girth (cm)			
Ankle girth (cm)			
Upper arm length (cm)			
Fore arm length (cm)			
Hand length (cm)			
Hand breadth(cm)			
Tibiale mediale sphyrion length(cm)			
Foot length (cm)			
Biacrimial (cm)			
Biiliocristal(cm)			
Transverse chest breadth (cm)			
Femur bi-epicondylar breadth (cm)			
Humerus bi-epicondylar breadth (cm)			
Body Composition			
Fat %			

Fat Mass(kg)			
Fat free Mass(kg)			
Total Body Water(kg)			
Lean Body mass(kg)			
BMR(kcal)			
BMI			
Skinfold (mm)			
Triceps			
Biceps			
Subscapular			
Supra iliac			
Supraspinale			
Abdominal			
Mid thigh			
Calf			

BIOCHEMICAL ASSESSMENT

Blood Haemoglobin : Serum Lactate Dehydrogenase :

Blood Glucose: Serum ferritin :

CLINICAL ASSESSMENT

Presence of Deficiency Symptoms **Clinical sign**	**Present / Absent**
Low body weight	
Night blindness	
Conjunctival xerosis	

Bitot spotCorneal Xerosis	
Keratomalasia	
Angular Stomatitis	
Clerosis	
Dental Caries	
Goiter	
Palpate	

APPENDIX IV

NUTRITION EDUCATION QUESTIONAIRE (KAP) METHOD

A. Knowledge related questions

1. Diet which contain all the essential nutrients is called

a) Balanced diet b) Nutritional food ingredients c) nutrients

d) Good nutrients

2. Colored vegetables and fruits contain

a) Vitamin b) Iron c) Fiber d) All of them

3. Which of the following fluid is the best for dehydration?

a) Oral rehydration solution b) Milk c) Water

d) Honey

4. Which mineral is lost during dehydration?

a) Sodium and Potassium b) Iron and Zinc c) Selenium and Copper

d) Only iron

5. Which of the following is the best source for treating constipation?

a) Dietary fiber b) Medicine c) Exercise d) Water

6. Which of the following is the best source of energy for sports person?

a) Carbohydrate b) Protein c) Fat d) Iron

7. Drinking sugar solution immediately before exercise

a) Enhance performance b) Delay fatigue c) Produce fatigue

d) Provide energy

8. Which of the following symptoms occur in dehydration?

a) Muscle Fatigue b) increase body weight c) soften bone

d) Increase blood volume

9. Which food does contain dietary fiber?

a) corn b) Refined flour c) White bread d) Meat

10. Which of the following nutrient is the most needed after exercise?

a) Carbohydrates and Protein b) only protein c) only Fat d) only carbohydrate

11. What do you think about the following statements?

Statements	Responses	
	Yes	No
For morning event ,the meal should be high carbohydrate at night and light breakfast at morning		
For afternoon event, the dinner at night and the breakfast in the morning should be high carbohydrate meals and the lunch should be light meals.		
For evening event, the breakfast and the lunch should be high carbohydrate meals followed by light meals.		
Vitamins and minerals are required in small quantities for proper functioning of the body.		
Minerals are needed in activating numerous reactions that release energy during the breakdown of carbohydrate, lipid and protein.		
A significant reduction in the dietary lipid can lead to reduced level of fat soluble vitamins.		

12. Do you think the following aspects help you to improve your sporting performance?

Aspects	Yes	No
Eating carbohydrate 2 to 4 hour before an event(positive statement)		
Taking multivitamin before an event (negative statement)		
Drinking water immediately before an event(negative statement)		
Eating protein during the event(negativestatement)		
Drinking coca cola during the event(negativestatement)		
Taking iron supplement without having iron deficiency (negative statement)		
Caffeinated drink during the event (negative statement)		

13. Are these hydration statements true or false?

Aspects	True	False
Athletes should consume 1.5 to 3 liter of fluid above their normal intake the day before the event		
Empty their bladder 15 minute prior to the event is a must		
Athletes should drink cold water during the event		
Athletes should sip the water ,and not gulf it down		
Athletes should consume 0.5 liter of water 1-2 hour prior to the event		
Athletes should consumed 0.6 liter of water \ fluid 10 -15 minute before the event		
Dark urine is an indication of dehydration		
Large amount of pale urine will be passed when fully hydrated.		

14. Attitude related question

Statement	Response				
	Strongly agree	Agree	Undecided	Disagree	Strongly Disagree
Lack of iron on the diet result in fatigue injury and illness(positive)					
Dehydration can impair physical performance					
The Nutritional needs of athletes differ from normal population(negative)					
Vitamin supplementation is recommended for physical active people					
Excess vitamin supplementation may harm the physical active person					
Fasting before training makes physiologically active throughout the day					
Consumption of raw egg is nutritious for physically active person					
High amount of water consumption will increase the body weight					
Dietary pattern should change with season and climate					
Excess amount of protein ingestion during exercise improve performance					
Natural Antioxidant supplementation helps in reducing oxidative stress during intense training					
Only drinking water before practice helps in performance					
Drinking milk immediately after exercise reduce fatigue					
Deficiency of B-group vitamins can result in premature fatigue and inability to maintain heavy training programme					

17. Practice related question

Statements	Responses			
	Always	Often	Sometimes	Never
Consuming raw egg				
Drinking only plain water before practice				
Consuming excess amount of protein				
Consumption of carbohydrate food 2-4 hour before event				
Drinking carbonated beverages				
Consume 0.5 liter of water 1-2 hour prior to the event				
Consuming high carbohydrate meals at night and light breakfast at morning as pregame meal for morning event.				
Emptying bladder 15 minute prior to the event.				
The dinner at night and the breakfast in the morning should be high carbohydrate meals and the lunch should be light meals for afternoon competition				
Consuming 0.6 liter of water \ fluid 10 -15 minute before the event				
For evening event, the breakfast and the lunch should be high carbohydrate meals followed by light meals				
Drinking cold water during the event				
Consuming 1.5 to 3 liter of fluid above the normal intake the day before the event				
Consumption of fiber rich food				
Consumption of fruits				
Consumption of varieties of foods in daily meals.				

APPENDIX V

ASSESSMENT OF BODY COMPOSITION

Principle:

Bioelectrical impedance measures the resistance of body tissues to the flow of a small, harmless electrical signal. The proportion of body fat can be calculated as the current flows more easily through the parts of the body that are composed mostly of water (such as blood, urine & muscle) than it does through bone, fat or air. It is possible to predict how much body fat a person has by combining the bioelectric impedance measure with other factors such as height, weight, gender, fitness level and age.

Procedure:

For predictive accuracy of the measurement, each individual should strickly followed BIA testing guidelines (Heyward and Stolarczyk (1996). before testing, the athletes were required to adhere to these BIA testing guidelines:

1. The athletes should not eat or drink within 4hour of the test

2. They should maintain normal body hydration

3. They should not consume caffeine or alcohol within 12 hour of the test.

4. Not to exercise within 12 hours of the test

5. Not to take diuretics within 7 days of the test

6) To urinate within 30 minutes of the test.

BIA measurement was taken by the Tanita Body Composition Analyser TBF300 A (made in Japan). The investigator asked the athletes to stand on the platform with their heels correctly aligned with the electrodes on the measuring platform. The Tanita analyzer measures lower-body resistance between the right and left legs as the individual stands on the electrode plates of the analyzer. Subjects were measured while standing erect, in bare feet, on the analyzer's footpads and wearing minimal clothing. The system's 2 electrodes are in the form of stainless steel foot pads. Leg-to-leg impedance and body mass are simultaneously measured as the subject's bare feet make pressure contact with the electrodes and digital scale.

APPENDIX VI

Estimation of Haemoglobin (Cyan Methaemoglobin Method)

Principle

Haemoglobin is converted into cyanmethaemoglobin by the addition of KCN and cyanide. The color of cyanmethaemoglobin is read in a photo calorimeter against a standard solution. Since cyanide has the maximum affinity for haemoglobin, this method estimates the total haemoglobin.

Procedure

5 ml of Drabkins solution was measured into a dry test tube from burette. Exactly 0.02ml of blood was transferred from a standard haemoglobin pipette into a dilutent solution. The blood and the dilutent are thoroughly mixed by rotating tubes. Ten minutes time was allotted for the formation of cyanmethaemoglobin, 5ml of the diluent solution was used as blank. With green filter 540mm. The readings were taken in the photoelectric colorimeter (Raghuramulu et al., 2003)

APPENDIX VII

ESTIMATION OF ENERGY EXPENDITURE RECORD

Name : Sex :
Age:
Occupation : Address
:

Time	(Activity type)	Total time	Similar Activity	Kcal/ minute
5.00 - 5.30 a.m 5.30 – 6.00 a.m 6.00 – 6.30 a.m 6.30 – 7.00 a.m				
7.00 - 7.30 a.m 7.30 – 8.00 a.m 8.00 – 8.30 a.m 8.30 – 9.00 a.m				
9.00 - 9.30 a.m 9.30 – 10.00 a.m 10.00 – 10.30 a.m 10.30 – 11.00 a.m				
11.00 - 11.30 a.m 11.30 – 12.00 p.m 12.00 – 12.30 p.m 12.30 – 1.00 p.m				
1.00 - 1.30 p.m 2.30 – 3.00 p.m 3.00 – 3.30 p.m 3.30 – 4.00 p.m				
4.00 - 4.30 p.m 4.30 – 5.00 p.m 5.00 – 5.30 p.m 5.30 – 6.00 p.m				
6.00 – 6.30 p.m 6.30 – 7.00 p.m 7.00 – 7.30 p.m 7.30 – 8.00 p.m				
8.00 - 8.30 p.m 8.30 – 9.00 p.m 9.00 – 9.30 p.m 9.30 – 10.00 p.m				
10.00 - 10.30 p.m 10.30 – 11.00 p.m				
Daily Total				

APPENDIX VIII

ASSESSMENT OF PHYSICAL PERFORMANCE

i) Cardio Respiratory Endurance :

It is the ability of the heart, lungs and circulatory system to supply oxygen and nutrients efficiently to working muscles. VO_2 max is the rate of oxygen utilization of the muscles during aerobic endurance in order to assess cardio respiratory endurance and functional aerobic capacity. Queen College Step test was used to measure of cardio respiratory endurance.

Equipment required:

16.25 inches, stop watch, metronome

Procedure:

The investigator asked the volunteer step up and down on the platform at the rate of 22 steps per minute for females and 24 steps per minute for male, for a total of 3 minutes. The athlete immediately stop on completion of the test and the carotid pulse is counted for 15 seconds from 5-10 second of recovery. The estimation of VO_2 max was calculated from the test results using the formula :

Man = VO_2 max(ml/kg/min)=111.33-(0.42x4x15 sec recovery pulse)

Female = VO_2 max(ml/kg/min)=65.81-(0.1847x4x15 sec recovery pulse)

ii. Speed

Speed is the trainable motor ability .It is defined as the capacity to move a limb or a body or any other part with greatest possible velocity. (Goswami, 2011). Speed of the athletes was assessed using 30 m flying start test.

Procedure: A total of 45 metres was marked at start and finish. In addition to these, another mark was drawn after 15 meter from the straight line. Three posts were erected, at start line and the distance beside the tract. The athlete runs all –out immediately of the receiving the start signal and should reach maximum speed by end of 15 m. The recorder note the time taken to run from the 15 m mark to the 45 m mark (a distance of 30 m).

iii) Explosive strength

Standing broad jump

Equipments: measuring tape, non slip floor, a stick to mark the landing point

Procedure:

The investigators mark a line before the landing pit. Then the athlete was asked to stand behind the marked line with fit slightly apart ,both feet are used for takeoff and landing .swinging of the arms and bending of the knees was also allowed

to provide forward drive .then the athletes was instructed to jump as far as possible ,landing on both feet and without falling backwards. Three attempts were allowed. The distance from the mark line and landing point is the score.

iv) Explosive power (ATP – CP based) of the leg muscles

Vertical Jump

Equipment used

Measuring tape or a marked wall, chalk for marking on the wall

Procedure: The athlete was asked to stand sidewise to a wall and reach up with the hand closest to the wall and keep the feet flat on the ground. Mark the point of the finger tips and record. Then the athlete was standing away from the wall and jump vertically as high as possible. He can use squatting down (counter movement) before jumping and take maximum assistance of using both arms and legs in projecting the body upwards. He should touch the wall at the highest point of the jump and make a mark with the chalk. The difference in distance between the standing reach height and the jump height is the score. The best of three attempts is normally recorded with about 30-60 sec break between two attempts.

v) Muscular Endurance

Press –ups or push-ups test

Equipment used

Mat placed on flat surface, A stop watch

Procedure

The subject was made to lie on the mat, hands, shoulder with apart and fully extend the arm, lower the body with the elbows reach 90^0. Return to starting position. The assistant (or partner player) places his fists on the ground in line with the performer's nipple line. The aim is to complete as many full push up in a minute as possible. The feet are not to be held together. Push up action was to be continuous with no rest and as many push-ups as possible should be completed. The number of full body part should be counted and recorded.

vi) Strength of the abdominal muscles

Sit- ups

Equipments used:

Mat place on a flat surface, stop watch, an assistant

Procedures

The athletes were oriented about the instruction. The event was demonstrated and common errors were pointing out. The athletes were made to lie on his or her back, knees bent, and heels flat on the floor. Hand was held behind the head, with elbow out to the sides. An assistant or partner held down the feet. The athletes were made to perform as many correct sit ups as possible in one minute. In the up position, the athlete was made to touch the elbows to the knees and then return to the lying position (shoulder blades touch the floor) before starting the next sit-up. The number of correct sit-ups was used as the score.

vii) Agility

Shuttle Run (6x10meter)
Equipments used:

Steel tape, two stop watches, 6x10 square marking.

Procedure

The 6x10 m shuttle run was used to test participant's agility. Two parallel lines were drawn on the tract 10 m apart. The athletes were instructed to be ready in position with one foot just behind the starting line. At the first signal, the participant ran as quickly as possible to the other line, crossing with one foot .He then turn round as quickly as possible and return to the starting line crossing it one feet. Skittles were placed at the turning points to ensure that the participant crosses the lines .The whole movement was repeated to make a total covered distance of 60 meters. The score was the time spent to complete the 60 meters and recorded in second.

APPENDIX IX

Estimation of pH

The best and widely used method of pH determination is by Measuring the electromotive force(emf) produced by H+ around an electrode. Here the basic principle is that when electrodes are kept in two solutions having differing H+ concentration and the electrodes are connected to a potentiometer and the solutions by a salt bridge, a smzll potential difference can be observed (emf). If one knows tha standard emf produced bya certain concertration of H+, the emf of other solutions can be measured by comparison. Electrmotive force is related to pH by the following equation:

$$pH: \quad \frac{emf}{0.00019837\ T}$$

Where T is the absolute temperature

The hydrogen electrode(platinum with platinum black) with hydrogen was at one atmospheric pressure and immersed in a1 N H+ solution is assigned a potential of zero, under all conditions and is used the standard reference electrode

Glass electrode

This method of determining pH is the widely ue=sed one at present the principle according to which the glass electrode functions is simple. It has been found him membrances of the proper glass are selectively permeable to H+ and if a cell is set as follows,

A	b	C	D
Ag----AgCI		unknown	HgCI--Hg
0.1 MHCI		soltion	saturated KCI
A	B	C	D

The (H+) and pH of an unknown solution may be determined. A-Repsents a silver-silver chloride in half cell containing 0.1M HCI, C- is the unknown solution to be tested, D is a standard calmel half cell and B is the glass membrane with a definite constant H+ activity on one side (0.1M HCI) and a varible H+ activity on the (unknown soltion)

$$PH = \frac{E - E'c}{0.00019837\ T}$$

Where E is the emf seen in unknown solution, E'c is the emf

Estimation of Total Sugars by Phenol Sulphuric acid Reagents

⇒ Sulphuric acid 96% reagent grade.
⇒ *Standard Glucose*: Stock – 100mg in 100mL of water. Working standard – 10mL of stock diluted to 100mL with distilled water.

Procedure

1. Weigh 100mg of the sample into a boiling tube.
2. Hydrolyse by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cool to room temperature.

3. Neutralise it with solid sodium carbonate until the effervescence ceases.
4. Make up the volume to 100mL and centrifuge.
5. Pipette out 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard into a series of test tube.
6. Pipette out 0.1 and 0.2mL of the sample solution in two separate test tubes. Make up the volume in each tube to 1mL with water.
7. Set a blank with 1mL of water.
8. Add 1mL of phenol solution to each tube.
9. Add 5mL of 96% sulphuric acid to each tube and shake well.
10. After 10min shake the content in the tubes and place in a water bath at 25-30°C for 20min
11. Read the color at 490nm.
12. Calculate te amount of total carbohydrate present in the sample solution using the standard graph.

Estimation of carbohydrate

Aim

To estimate the carbohydrate content of the given food sample

Principle

Carbohydrate are first hydrolysed in to simple sugar using dilute HCl. In hot acidic medium, glucose is dehydrated for hydroxy methyl furfural. This compound forms with anthrone green coloured product with an absorption maximum at 630 nm.

Materials

- 2.5 N – Hydrochloric acid
- Anthrone reagent : Dissolve 200 mg anthrone in 100ml of ice cold 95% H2SO4 prepare fresh before use
- Standard glucose stock: Dissolve 100 mg of glucose in 100ml water
- Working standard: 10 ml of stock diluted to 100 ml with distilled water, store refrigerated after adding a few drops of toluene.

Procedure

- Weigh 100 mg of the sample into a boiling tube
- Hydrolyse by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5N HCl and cool to room temperature
- Neutralise it with solid sodium carbonate until the effervescence ceases.
- Make the volume to 100 ml and centrifuge
- Collect the supernatant and take 0.5 and 1 ml aliquots for analysis
- Prepare the standards by taking 0.2, 0.4, 0.6, 0.8 and 1ml of working standards and ‘0’ as blank.

- Make the volume of 1 ml in all the tubes including the sample tubes by adding distilled water.
- Then add 4 ml of anthrone reagent
- Heat for 8 minutes in a boiling water bath
- Cool rapidly and read the green to dark green colour at 630nm
- Draw a standard graph by plotting concentration of the standard on X-axis Vs absorption on Y – axis
- From the graph calculate the amount of carbohydrate present in the sample.

Determination of Sodium and Potassium content in food stuffs by Atomic Absorption Spectrophometer

Weigh, to the nearest 0.1 mg, a test sample portion of 200 to 300 mg into the teflon crucible of a pressure decomposition vessel. For safety, the decomposition vessel should never be charged with 350 mg of sample. Add 5.0 ml of nitric acid to the test portion, place the teflon crucible in the stainless steel bod, close with the teflon lid and scew-on the stainless cap and close hand tight. Heat the vessels during 10 hours at 15Oº C in an oven maintained at this temperature. Remove the vessels from the oven, unscrew the cap and transfer the contents of the teflon crucible, including drops adhering at the inner side of the lid, to a 100 ml volumetric flask. Wash crucible and lid with water and combine these washings with the contents of the volumetric flask. Use approximately 50 ml of water to transfer the digest and to wash the teflon crucible. Add 4.0 ml of the cesium solution and 0.2 ml of the bromocresol green solution to the diluted digest, use adjustable pipettes to do this, and mix. Add ammonia solution to change the colour of the indicator from yellow, via green, to just blue and swirl. Dropwise add hydrochloric acid until the colour of the solution just remains yellow, pH 4, dilute with water to volume and mix.

Blank test - Carry out a blank test using the same conditions as in the test portion by 0.3 ml of water.

Determination

Sodium measurement

Select the appropiate settings and conditions for the flame atomic absorption measurement of sodium, lean-blue air-acetylene flame, and adjust the spectrometer to obtain optimal measuring conditions for sodium at 589.6 nm wavelength. Zero the instrument with water and aspirate the blank test solution obtained and the test solution. Adapt, if required, the optical pathlength in the flame and record the instrument readings. The sodium content of the test solutions as adequately as possible and aspirate as indicated. Dilute, if required, the test solutions with cesium solution in a screw-cap bottle using adjustable pipettes and carry out the sodium measurement as specified above.

Draw the calibration graph for the standard solutions selected and correlate the readings of the test solutions with the corresponding sodium content of the solution. Under optimal conditions the readings of the blank test solution and the zero standard solution should be identical. Select a set of standard sodium/potassium solutions covering the sodium content of the test solution as adequately as possible and aspirate as indicated.

e. Potassium measurement - Carry out the potassium measurement as described under substituting sodium for potassium. Set the instrument at 766.5 nm wavelength for this element.

RESULTS

Methods of calculation and formulas

- The sodium content, expressed in percent by mass, of the product is given by the formula: c_1,xf_1X10/ m

where c_1 is the sodium content of the test solution in milligrams per litre, read from the calibration graph.

f_1 is the dilution factor of the test solution

m is the mass, in milligrams, of the test portion

Report the results in three significant decimal places. The potassium content, expressed in percent by mass,of the product is given by the formula:

C_2 x f_2 x10 /m

where

c_2 is the potassium content of the test solution, in milligrams per litre, read from the calibration graph.

f_2 is the dilution factor of the test solution

m is the mass, in milligrams, of the test portion

The results can be reported in three significant decimal places.

Estimation of Iron

Principle

Iron is determined colorimetrically with ferric ion which gives a blood red colour with potassium thiocyanate.

Reagents

Stock Iron Solution : Dissolved 0.702 gm of reagent grade crystalline ferrous ammonium sulphate (Mohr's salt) in 1000 ml of water.

Working Standard : Prepared a working standard in 100 ml volumetric flask by adding 10 ml of the stock iron and diluted to the mark with distilled water.

Saturated potassium persulphate solution : Shook 7.8 gm of the reagent grade potassium persulphate in 100 ml water in a glass stoppered bottle. The undissolved crystalline potassium persulphate settle to the bottom and compensated the loss by decomposition.3Npotassium thiocyanate –dissolve 146 g of potassium thiocyanate to 500 ml in water.

Procedure

Two ml of conc H2SO4 in a 50ml volumetric flask.Add exactly 0.5ml of well mixed blood and add to this flask,2ml of potassium persulphate,agitate the flask,cool and dilute with about 25ml distilled water.Then 2ml sodium tungstate is added and the volume made up to the mark. Filter using whatman No 42 filter paper.Transfer 15ml of the filterate to a fresh tube,add 1ml of potassium persulphate and 4ml of potassium thiocyanate.Mix and read the colour at 540nm.A standard (10-100Pg)is run similarly and a standard graph is prepared.

Estimation of Vitamin – C

Ascorbic acid reduces the 2,6-dicholorophenol indophenol dye to a colourless lecuo-base. The ascorbic acid gets oxidised to dehydro ascorbic acid. Though the dye is pink coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium. Oxalic acid is used as the titration medium.

Materials

• 4% oxalic acid

• Dye solution: 42mg of sodium bicarbonate was dissolved in a small volume of distilled water. 52 mg of 2, 6-dicholoro phenol indophenol was dissolved in it and made up the volume to 200 ml with distilled water.

• Stock solution: 100 mg in 100 ml of 4% oxalic acid

• Working standard: 10 ml of stock was diluted in 100 ml of 4%oxalic acid. Concentration of working standard was 100 Pg / ml.

Procedure

5 ml of the working standard was pipette out in a 100 ml conical flask.10 ml of 4% oxalic acid was added to the working standard solution and titrated against the dye (V1ml). End point is the appearance of pink colour which was persisted for few minutes. The amount of the dye consumed was equivalent to the amount of ascorbic acid.5g of the sample was extracted by addition of 4% oxalic acid and the supernatant was collected.5ml of supernatant was pipette out and 10 ml 4% oxalic acid was added and titrated.

Calculation

$$\text{Amount of ascorbic acid mg /100 g sample} = \frac{0.5 \text{ mg} \times V2\text{ml} \times 100 \text{ ml X } 100}{V1\text{ml X } 5 \text{ X wt of the sample}}$$

Total Antioxidant Assay by Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the methods of Benzie and Strain (1999) with slightly modification. An amount of 200 µl extracted samples were mixed with 3 mL FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37oC and the absorbance of the samples was determined against blank at 593 nm. Series of stock solution at 200, 400, 800, 1200 and 1600 µM were prepared (r2= 0.9944) using aqueous solution of FeSO4.7H2O as standard curve. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of freeze dried sample.

APPENDIX X

PROCEDURES USED FOR MICROBIAL ANALYSIS SERIAL DILUTION TECHNIQUE

Aim
To dilute the sample and find the microbial load

Equipments
Incubator, laminar air flow, autoclave, Bunsen burner

Materials
Sample, sterile distilled water, conical flask, test tubes, etc.,
Procedure
1. Using separate sterile pipettes make decimal dilutions of 10-2, 10-3, 10-4, 10-5 and 10-6
2. Add 10ml of seaweed homogenate to 90ml of diluent it is 10-2 dilution
3. Serially dilute in flasks containing 90 ml of diluent and soon up to 10-6

Total Bacterial Count

Aim

To enumerate the total microorganisms in seaweed samples.
Equipments
Incubator, laminar air flow, autoclave, Bunsen burner

Materials
Nutrient agar, pipette, conical flask, petridish, etc.,

Procedure
1. Pipette out 1ml from each dilution into separate sterile petridish.

2. Add 15 ml of the molten nutrient agar in each petridish.
3. Mix the inoculum with the agar by swirling the plate.
4. Allow to solidify for 30 minutes.
5. Incubate at 370C for 24 - 48 hours.
6. Count the colonies and select the plates containing not more than 300 colonies and not less than 15 colonies in two consecutive dilutions.

Calculate using the formula

$$\frac{\pounds C}{(n1+(0.1*n2))d}$$

Where

$\pounds C$ = sum of the counts frown both dilutions

n1 = number of plates counted from the first dilutions

n2 = number of plates counted from the second dilutions

d = dilution factor of which is used as lower dilution

APPENDIX XI

Estimation of Blood glucose

Principle

Glucose is oxidized to gluconic acid by glucose oxidase. The hydrogen peroxide liberated is reduced by peroxidase and the oxygen transferred to an acceptor, which is colourless in the reduced form but coloured in the oxidized form.

Reagents

a. Protein precipitant:
   Sodium tungstate. $Na_2Wo_4H_2O$ - 10g
   Disodium phosphate Na_2HPO_4 - 10g
   Sodium chloride - 9g

   Dissolve the above in about 800ml water and add approximately 125ml of 1N HCL to adjust to pH 3.0. add 1g of phenol and make up to 1L with water. Stable for 1 year at 25^{o}C.

b. Colour reagent:
   Sodium azide - 0.3g
   4-aminophenazone - 0.1g
   Disodium phosphate - 3.0g

Dissolve them in 295ml of water, then add 5ml of glucose oxidase – peroxidase mixture. The mixture should have at least 1.5 µU and 3.0µU/ml of glucose oxidase and peroxidase respectively. Stable for 8 week at 4°C.

c. Standard

The stock standard contains exactly 1g of pure glucose in 100ml benxzoic acid solution. The working standard is made by diluting 1ml of stock standard with 49 ml benzoic acid solution. This solution is stable at 25^0C for one year.

PROCEDURE

1. Pipette 0.1ml of blood into 2.9 ml of protein precipitant. Mix well and centrifuge for about 5 minutes. A standard curve is set up for each batch of determinations. Into clean tubes pipette 0.1, 0.2 and 0.3 ml of the working glucose standards equivalent to 60,120 and 180 mg per 100 ml. In each case, make up the volume to 1ml with protein precipitant reagent.

2. Similarly, take 1ml of clear supernatant from the test and place in clean tubes. For the reagent blank, use 1ml of protein precipitant. To all tubes, add 3ml of colour reagent and incubate at 37^0C for 10 minutes. Then place the tubes in cold water for 1 minute and read the absorbance at 505 nm against the reagent blank without further delay.

3. Plot the absorbance of the standards graphically and read of the glucose values of the test from this. If the glucose concentration of the test is greater than 180mg per 100ml, repeat the colour development stage using a smaller aliquot of the supernatant example 0.2 or 0.5 ml. Include additional standards n the calibration where necessary and multiply the result obtain by the appropriate factor, depending on the volume of supernatant use.

Estimation of Serum Ferritin

1. Place empty micro sample cups onto 16 X 100 tubes in a test tube rack and pipette 150 µL of the serum samples into the sample cup. Be sure to load the samples from left to right.

2. For a calibration run: Pipette 500 µL of calibrator into the sample cup, and place in designated calibrator positions on the inner wheel.

3. For runs other than calibration run: Pipette 150 µL of all levels of control into each sample cup, and place in designated control position on the inner wheel. Pipette 150 µL of patient sample into each sample cup and place on the instrument in the

order of the work list starting with position 1 and ending with position 50 for the first run.

4. Ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles.

5. Ensure that the amount of reagents, diluent, and wash solutions is adequate for the amount of samples to be run. You may place more than one bottle of reagent at a time on the analyzer however; avoid using more than one lot number of reagent for a single run.

6. The method principle for measurement of Ferritin is immuno-turbidimetry using Roche kits on the Hitachi 912 clinical analyzer.

Estimation of Serum Lactate DHASe

LDH assays can be performed by assessing LDH released into the media as a marker of dead cells or performing lysis LDH as a marker of remaining live cells. Prepare Lactate Dehydrogenase Assay Mixture fresh for each experiment: Mix equal amounts of Lactate Dehydrogenase Assay Substrate, Assay Dye and enzyme in order to have 20 µL for each well that will be measured plus 10% extra for error. Enzyme aliquots are in the -20ºC freezer and are in 400 µL and 750 µL volumes. 400 µL is sufficient for one plate; 750 µL is sufficient for 2 (24) well plates. The Substrate and Dye are in the tissue culture refrigerator. The enzyme must be kept in the dark, and the reagents are added in the dark, with the hood light off.

1. In a 96 well clear plate, fill all wells in column A with 40 µL MEM/BSA/Hepes (+/- N2 if appropriate) as blank.
2. Pipette 40 µL of each sample from your toxicity experiment in duplicate into the 96 well plate. Using a repeater, add 20 µL of the substrate/dye/enzyme mix to each well. (Pop any bubbles with a needle/forceps)
3. Cover the plate with a paper towel and bring it to the plate reader outside the cold room.
4. Open the Magellan program on the desktop. Use the manual setting to shake the plate for 5 seconds, and then store it in a dark location (inside the shelf under the laptop) for 20-30 minutes at room temperature.
5. Use the Magellan program on the plate reader outside the cold room to measure absorbance at a wavelength of 492 nm.

PUBLICATION

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