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METHODOLOGY

The present study titled "**Effect of Interventions on Nutritional Status and Work Performance of Thang - Ta Athletes in Manipur**" was carried out under the following headings:

PHASE I

COLLECTION OF BACKGROUND INFORMATION

A. Selection of Area and Athletes

B. Development of Methods and Tools for Data Collection

C. Assessment of Socioeconomic Status

PHASE II

ASSESSMENT OF NUTRITIONAL STATUS AND PHYSICAL PERFORMANCE

A. Nutritional Anthropometry

B. Blood Haemoglobin Estimation

C. Clinical Examination

D. Dietary Survey

E. Physical Performance

PHASE III

INTERVENTIONS AND IMPACT EVALUATION

A. Supplementation with *Hibiscus sabdariffa Linn* juice (Hibisa)

1. Formulation and Standardization
2. Acceptability Trials among Athletes
3. Nutrient Analysis of Hibisa
4 Administration of Hibisa
5 Evaluation of the Effect of Supplementation

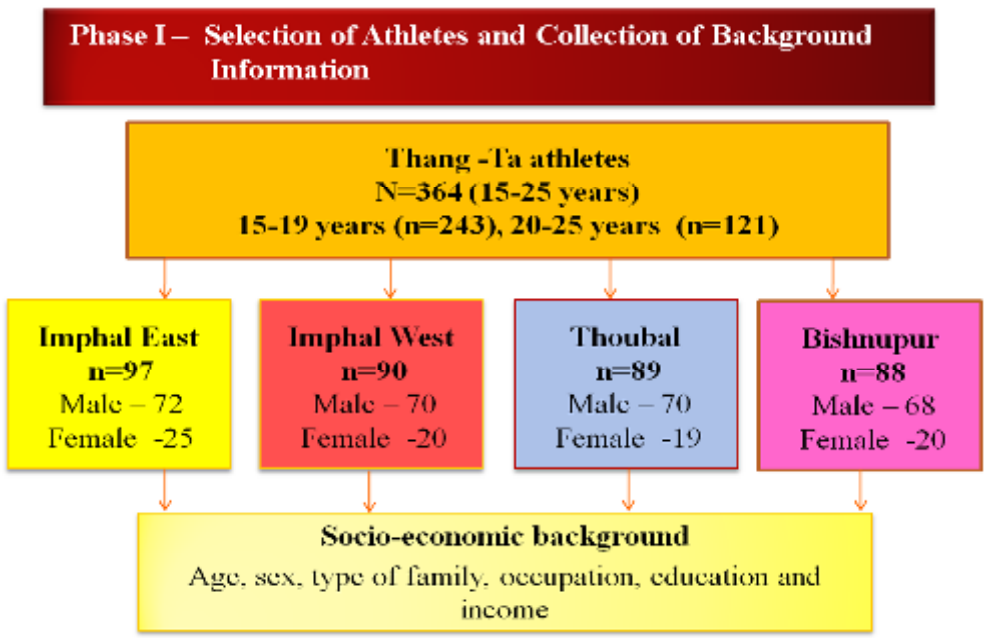
B. Formulation of Nutrition Education Material, Imparting Nutrition Education and Impact Evaluation

1. Formulation of Nutrition Education Materials
2. Imparting Nutrition Education
3. Evaluation of the Impact of Nutrition Education

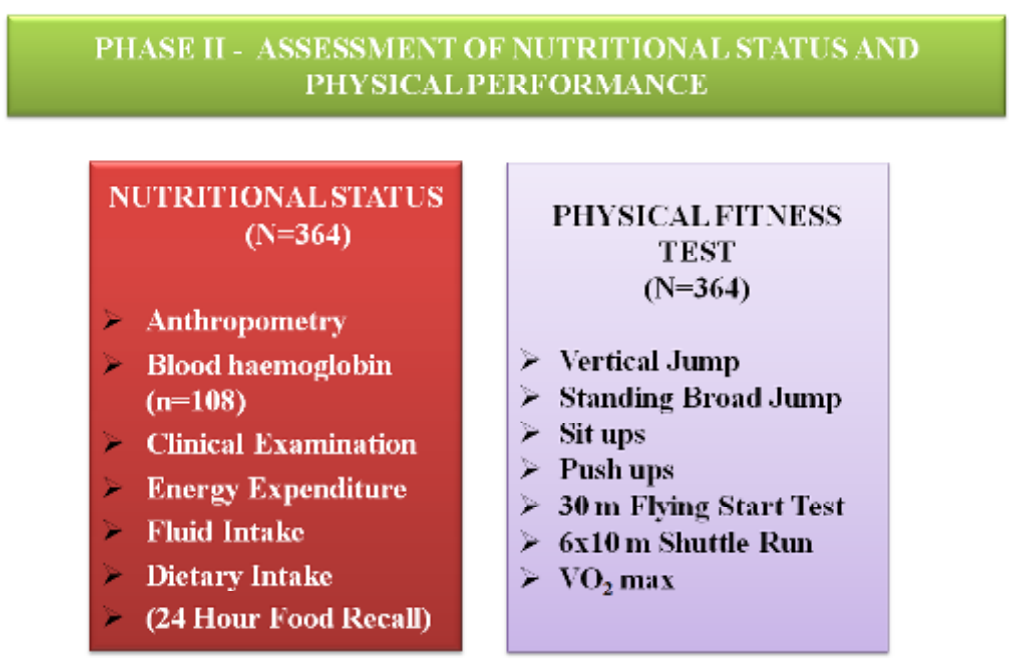
The Research Design of the Methodology followed for the study is given in Figure1.

RESEARCH DESIGN

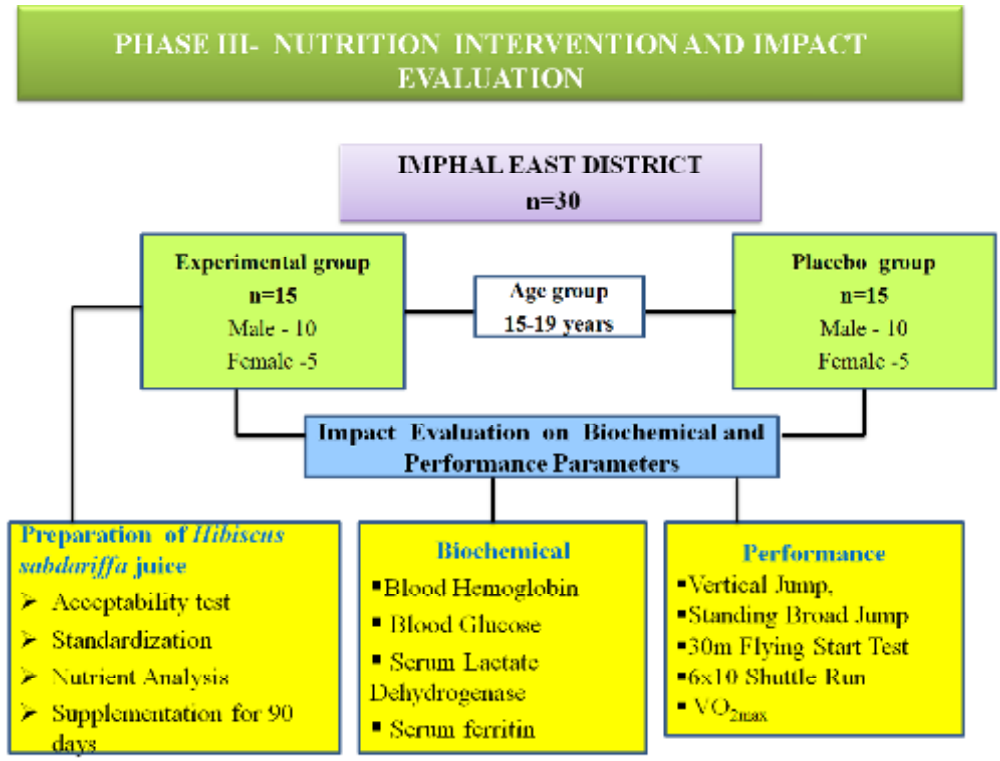
PHASE I



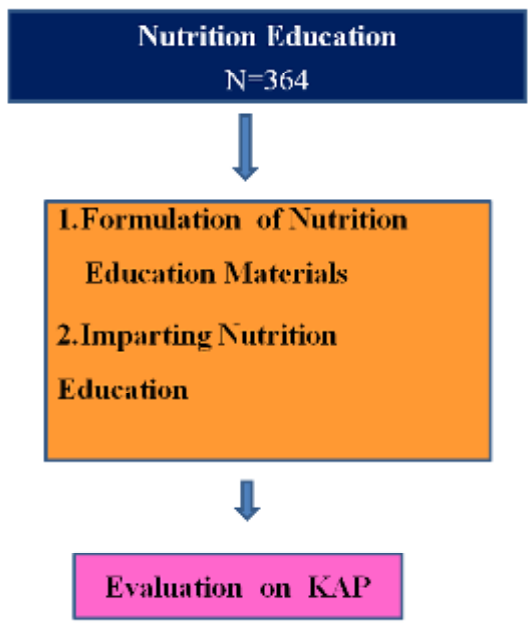
PHASE II



PHASE III



PHASE IV



Research Design

Figure 1

PHASE I

COLLECTION OF BACKGROUND INFORMATION

A. Selection of Area and Athletes

The present study was carried out in the state of Manipur. Totally Manipur has nine districts. Only four districts namely Imphal east, Imphal west, Thoubal and Bishnupur district were chosen since indigenous Thang-Ta athletes were more confined to these areas as they are valley districts, with easy approachability and accessibility. Figure 1 shows the Map of Manipur depicting the selected areas.

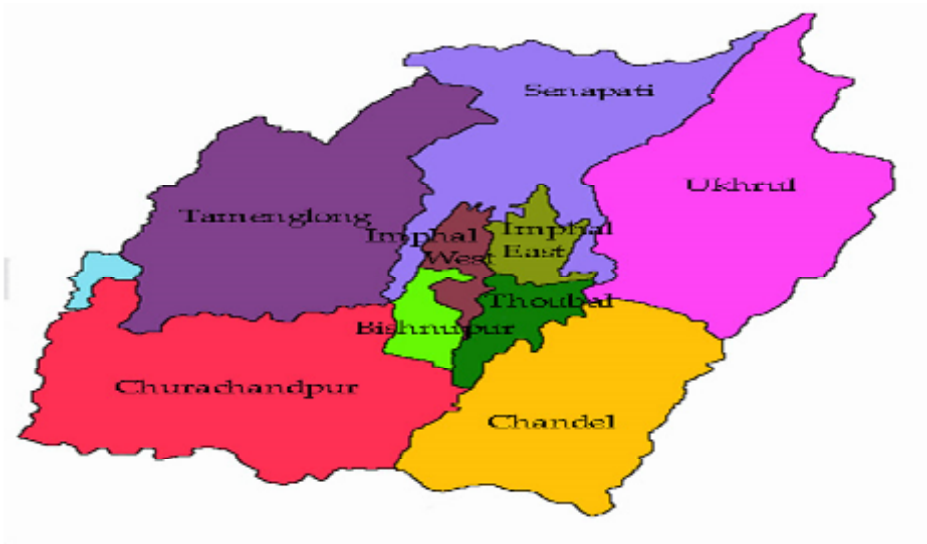


Figure 2: Map of Manipur

From each district, one main training center was selected. These centers had more number of students who were regular and students from other small centers could be gathered here. The athletes were selected using purposive sampling method (Kothari, 2005), in which method the desired number of sample units are selected deliberately or purposively depending upon the object of enquiry (respondents between 15-25 years of age) so that only the important items representing the true characteristics of the population were included in the sample.

Totally, 364 (280 male and 84 female) Thang -Ta athletes constituted the total sample size for the study. Using convenient sampling technique, 72 male and 25 female Thang - Ta athletes were selected from Huyen Lallong Manipur Thang -Ta–cultural Association, Irilbung (Imphal East district), 70 male and 20 female athletes were selected from Huyen Lallong Thang-Ta Academy, Yumnam Huidrom (Imphal

West District), 70 male and 19 female athletes from Association for Paona Memorial Art and Rural Development Services Thoubak Wangkhem (Thoubal District) and 68 male and 20 female athletes from Divine Cultural Thang -Ta Academy (Bishnupur District).

Before conducting the study, approval was obtained from the Institutional Human Ethics Committee, Avinashilingam Institute for Home Science and Higher Education for Women (AUW/IHEC-13-14/FSN-41), Coimbatore and the Local Human Ethics Committee from Manipur University (Ref.no.Ac/IHEF/MU/201/2014) (Appendix I).The investigator took permission from the authorities of the selected training centers and the willingness from each of the athletes (Appendix II). One hundred and ninety-eight male athletes were adolescents (15-19 years) and 82 were young adults (20-25 years). Similarly, 45 female athletes in the age group of 15-19 years and 39 were in the age group of 20-25 years.

Socio economic status, nutritional anthropometry, clinical status, dietary survey (24 hour food recall) and physical fitness level were determined for all the athletes. Blood haemoglobin levels were assessed on 108 willing athletes.

Selection of Athletes for Nutrition Intervention

1. Supplementation

Thirty athletes (20 male and 10 female) who were involved in regular practice and were willing to take part were included in the supplementation study .These athletes were then divided into two groups (experimental and placebo groups) of 15 (10 male and 5 female) athletes each by lottery method.

Sample size calculation: Only the willing athletes were included in the supplementation study. It was very difficult to get the appropriate or large sample size therefore it was not possible to calculate for achieving appropriate effect size for the proposed study**.**

2. Nutrition Education

Nutrition education was provided to all 364 athletes included in the study.

B. Development of Methods and Tools for Data Collection

Scheduled questionnaires are particularly useful in getting the correct information, because the investigator can explain the questions thoroughly to the

respondent through face to face contact and clarify doubts (if any) raised by the respondent in the course of collecting information (Kothari, 2004).

Scheduled questionnaire method was applied to collect data from the athletes. Questionnaire was formulated to assess Socio-Economic Status (SES) of the athletes which included detailed information on aspects such as income, type of family, occupation etc (Appendix III).

A separate questionnaire was modified and structured to assess the nutritional status. The aspects of nutritional status assessment included anthropometry, biochemical, clinical assessment, medical history, dietary pattern, life style pattern question. Dietary intake was assessed by 24 hour recall method by using ICMR Proforma.

Nutrition education questionnaire was formulated by referring Nutrition and Hydration Guidelines for Excellence in Sports, ISLI, NIN and SAI (2007) on aspects regarding basic nutrition and sport nutrition Knowledge, Attitude and Practice (Appendix IV).

C. Assessment of Socio economic Status

Socio economic status of all the 364 athletes was assessed in term of age, sex, type of family, religion, area of residence, family income, educational status and occupation using the questionnaire to assess socio economic status.

PHASE II

ASSESSMENT OF NUTRITIONAL STATUS AND PHYSICAL PERFORMANCE

A. Assessment of Nutritional Anthropometry

Nutritional anthropometry is concerned with the measurement of the variations of the physical dimensions and the gross composition of the human body at different age levels, activity and degree of nutrition (Jelliffe, 1966).

Anthropometric parameters

Height and weight; skin fold thickness such as biceps, triceps, subscapular, supraspinal, supra iliac, abdominal, front thigh, and medial calf;Body circumference – chest, waist, abdominal, hip, mid- thigh, upper arm relax, upper arm contract, fore

arm, wrist and ankle; Body diameter such as biacromial, biiliocristal, transverse chest breadth, Humerus Biepicondylar,Femur Bicondylar; Length such as upper arm, fore arm, hand, foot length were measured based on Sport Kinanthropometric Assessment Guidelines for Athletes (http:// homepages.ihug.co.nz/~rip/ Anthropometry/). The anthropometric sites and descriptions provided by Ross and Jones (1991) and the standard specifications of the International Society for the Advancement of Kinanthropometry (ISAK) were taken into consideration for measurement of anthropometry using the appropriate equipment and standard procedures.

Instrument used in the measurement: Herpenden skinfold caliper, brand name Herpenden, EAN-0634746665171, Part Number-C-136, UPC-634746665171 use for measurement of skinfold thickness. Tainita Body Composition Analyser TBF-300 A made in Japan use for measurement of body composition and Herpenden anthropometer, brand name Holtain, Model 601 made in United Kingdom for use for the measurement anthropometry.

Technical error measurement: Each anthropometric parameter was measured thrice and the average of the three measurements was calculated and taken as the mean value. The instruments were calibrated with known weight each day before taking the measurements.

a. Standing Height

Height of an individual is influence both by genetic and environmental factors. Height is affected only by long term deprivation. It is considered an index of chronic or long deprivation (Srilakshmi, 2010).

Standing height was measured using a height stadiometer. The athletes was made to stand erect on the platform of the stadiometer, facing directly ahead without shoes, feet together and arm hanging by the sides. The measurement was taken as the maximum vertical distance from the platform of the stadiometer to the vertex of the head, firmly touched by the sliding head pointer of the stadiometer and was read from the calibrated scale.

b. Body weight

Body weight is the most widely used and reproducible anthropometric measurement for the assessment of nutritional status. It indicates the body mass

and is a composite of all body constituents like water, minerals, protein bone etc (Bamji *et al*., 2004).

For the present study, body weight was measured by using TANITA Body composition analyser TBF 300A.The athletes were made to wear minimal clothing and he/she did not hold any other object for support and without foot wear while standing on the platform of the TANITA analyser. The body weight of the subject was automatically measured when the athletes stand on the foot plate of the analyzer

c. Skinfold Measurement

Herpenden skinfold caliper was used to measure the skinfold thickness of the Thang -Ta athletes at

i) Biceps skinfold

The biceps skinfold was measured over the biceps muscle in the middle of the upper arm mid part of the distance between inferior border of the Acromian process and the external superior border of the head of the radius (Plate 1a).

ii) Triceps

The athlete was made to stand erect with the hands hanging down by the side. The skin fold was picked up at triceps muscles of the right arm, mid way between the acromion process and the superior border of radius in line with the olecranon process. The fold was kept parallel to the length of the arm (Plate 1b).

iii) Supra iliac

The athlete was made to stand, arms down at the sides, the skin fold at the side of the waist, just above the line crest was grasped. The caliper was applied and the spring handle was fully released. The skin fold thickness was recorded to the nearest 1mm when the drift of the scale was ceased (Plate 1c).

iv) Supraspinale

The calipers was applied 1 cm anteriorly from the left thumb and index finger raising a fold at the intersection of the border of the ilium and a line from the spinal to

the anterior axillary border .The fold follows the natural fold lines running medially downwards at about a 45 degree angle from the horizontal.

v) Subscapular

The fat fold was measured just below and lateral to the angle of the left scapula by picking it up with thumb and fore finger in a line running approximately 45^0 to the spine, in the natural line of skin cleavage.

The athlete was made to stand relaxed with arms down at the sides. The skin fold was grasped at the lower tip of the shoulder blade with crease being at about 45 degree angle and the spring handle was fully released and the skin fold thickness was recorded to the nearest 1 mm when the drift of the scale was ceased (Plate 1d).

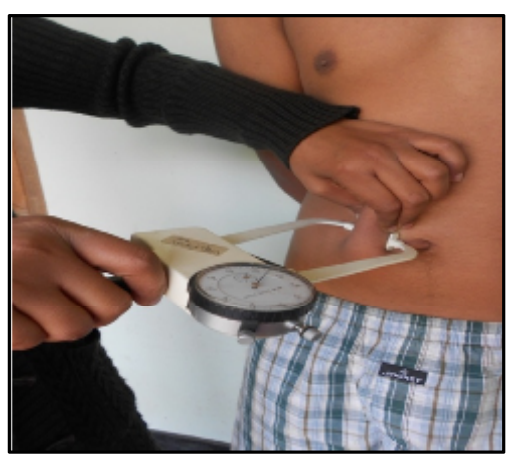
a. Biceps Skinfold

b. Triceps

c. Supra iliac

d. Subscapular

e. Medical Calf



f. Abdominal

Plate 1. Skinfold Thickness of Thang – Ta Athlete

vi) Medial calf Skinfold

The athlete was asked to sit with his knee bent at right angle. The skin fold was picked up on the medial side of the right calf slightly above the level of the maximum girth. The fold was held parallel to the long axis of the leg (Plate 1e).

vii) Abdominal skinfold

The caliper was applied 1 cm inferiorly to the left thumb and index finger, raising a vertical fold on the right side 5 cm lateral to, and at the level of the omphalion (midpoint of the navel) (Plate 1f)

viii) Front thigh skinfold

The caliper was applied 1 cm distally to the left thumb and index finger, raising the fold on the anterior of the right thigh, along the long axis of the femur, when the leg was flexed to a 90° at the knee by placing the foot on a box. The mid-thigh position for this measure was the estimated half-distance between the inguinal crease and anterior patella, where the skinfold was measured.

d. Diameter

i. Humerus bi-epicondylar diameter

The athlete was asked to stand and the width between the medial and lateral epicondyl of the humerus, with the shoulder and elbows flexed to 90 degree was measured. The caliper was applied at an angle approximately bisecting the angle of the elbow, with firm pressure of the cross bar in order to compress the subcutaneous tissue (Plate 2a).

ii. Femur bi-epicondylar diameter

The athletes was asked to sit with knee bent at right angle and the greatest distance between the lateral and medial bicondyles of femur was measured with firm pressure on the cross bar in order to compress the subcutaneous tissue (Plate 2b)

iii) Biacromial

It is the horizontal distance between the acromion of shoulder (Taylor and Francis, 1998). The athlete was made to stand erect and the horizontal distance across the shoulder was measured between the acromia (Plate 2c).

a. Humerus bi-epicondylar

b. Femur bi-epicondylar

c. Biacromial

d. Biiliocristal

e. Transverse chest breadth

Plate 2. Anthropometric Body Diameters

iv) Biiliocristal

It is the distance between the most lateral points on the superior border of the iliac crest. The investigator stood in front of the athlete, located the sites with his/her third digits and applied the branches of the anthropometer used as a sliding caliper to the sites. The branches of the caliper point upwards at an angle of about 45° from the horizontal to encompass the largest diameter between the lateral aspects of the iliac crests. Firm pressure was applied to the branches over the iliac sites by the investigator's index finger (Plate 2d).

v)Transverse chest breadth

It is the distance of the lateral aspect of the thorax at the level of the most lateral aspect of the fourth rib. This was obtained by applying the anthropometer used as a sliding caliper to the athlete who was seated erect and faced by the investigator. The caliper was applied at an angle of about 30° downward from the horizontal avoiding both the pectoral and latissimus dorsi muscle contours. When the site was approximated, the investigator removed the thumbs from the pinch grasp of the branches and applied firm pressure with the index fingers. The measurement was made at the end of the normal expiration (Plate 2e).

e. Circumference

i) Chest circumference

The athlete slightly abducted his/her arms to permit the anthropometrist, standing to the subject's right facing him/her, to pass the tape around the chest; the tape is held in position of with the right hand while the anthropometrist's left hand adjusts the tape at the athlete's back to the horizontal level of the marked mesosternale. Cross-handed technique was used to put the tape scale in juxtaposition with the zero on the stub end of the tape. The reading was obtained at the end of a normal expiration (Plate 3a).

ii) Waist circumference

The athlete was made to stand erect. The appraiser used cross handed technique to position a non stretchable fiber glass tape horizontally at the level of noticeable waist narrowing. The tape was then placed in the recording position and the measurement was made (to the nearest millimeter) at the end of the normal expiration (Plate 3b).

iii) Hip circumference

Hip circumference was measured in the horizontal plane at the level of maximal circumference including the maximum extension of the buttocks, posteriorly.The athlete was made to stand erect in front of the investigator with feet together, the tape was positioned around the hip, at the level of symphysis pubis and the greatest gluteal protuberance and the circumference was measured using fiber glass tape up to 0.1 accuracy at the fullest part of the hip (maximum posterior of the extension between the iliac crest and buttocks) keeping the tape parallel to the floor according to Bender (2005) and recorded.

a. Chest circumference

b. Waist Circumference

Plate 3. Body Circumference

Waist to Hip Ratio (WHR)

Waist hip ratio gives the distribution of fat in the human body. A waist hip ratio greater than 1.0 in men and 0.8 in women is an indicator of increased the risk of artherosclerosis (Srilakshmi, 2010). The standard waist to hip ratio is 0.8 in women and 1.0 in men and formula for calculation of WHR is shown below:

$$\text{WHR} = \frac{\text{Waist Circumference (cm)}}{\text{Hip Circumference (cm)}}$$

iv) Mid thigh circumference

This girth measurement is usually taken on the right side of the body. The athlete was made to stand erect with their weight evenly distributed on both feet and legs slightly parted. The site to be measured was marked. The circumference measure was taken at the level of the mid-point, on the lateral surface of the thigh, mid way between the top of thigh bone, femur and tibia lateral (top of the tibia bone). When recording, it was ensured that the tape was not too tight or too loose and lie flat on the skin horizontally.

v) Abdominal circumference

The abdominal circumference was taken at the level of the umblical groove (belly-button). While measuring, the tape was not too tight or too loose and was lying flat on the skin horizontally.

f. Length

i) Upper arm length

It is distance from the marked acromiale to the marked radiale. The athlete was made to stand erect with the arms at the sides, palms against the thighs. Anthropometer was used to measure the length (Plate 4a).

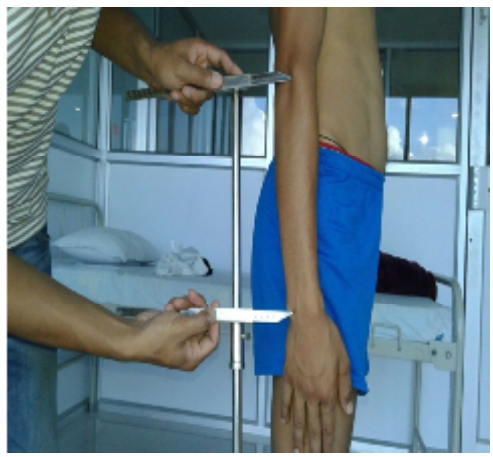
ii) Fore arm length (Radiale - stylion)

It is the distance from the marked radiale to the marked stylion. The investigator allowed the athlete to flex the elbow and by using anthropometer the distance was measured (Plate 4b).

iii)Hand length (Midstylion - dactylion).

It is the distance from the marked mid-stylion (at the distal wrist crease) to the dactylion. The athlete were asked to extend the right hand supinated, fully extending the fingers. By using the end pointer of sliding caliper was placed on the marked mid-stylion line, the housing pointer applied to the dactylion and the length was measured (Plate 4c).

a. Upper Arm Length



b. Fore Arm length

c. Hand length

d. Thigh length

e. Tibial length (Tibiale mediale – Sphyrion tibiale)

Plate 4. Body Length

iv) Foot length

It is the distance between the akropodion and pternion obtained by the anthropometer used as a sliding caliper on the standing athlete. The caliper was held parallel to the long axis of the foot. The investigator held the branch end of the caliper in the left hand, grasped the shaft with the right hand digits 2,3,4;in opposition to digit 5; while manipulating the cursor with the thumb. The sites were encompassed with minimal pressure and the length measured

v)Thigh length (Trochanterion - tibiale laterale).

It is the distance from the marked trochanterion to the marked tibiale laterale. The investigator asked the athlete to stand with feet together on the box with the right leg facing her, and then with the help of anthropometer, the thigh length was measured (Plate 4d).

vi) Tibial length (Tibiale mediale –Sphyrion tibiale)

It is distance from the marked Tibiale medial to marked Sphyrion tibiale. The investigator asked the athlete to sit on the box and across the right angle over the left knee to present the medial surface of the right leg horizontally. The end pointer was applied to the marked Tibiale medial and the housing pointer is extended to the marked Sphyrion tibiale (Plate 4.e)

2. Assessment of Body Composition

Body composition was measured with the help of body composition analyser (TANITA 300 A, Japan). The analyzer measured fat mass, fat free mass, fat per cent, TBW, BMR, BMI, and Impedance. The Procedure of body composition Assessment is appended (Appendix V)

B. Blood Haemoglobin Estimation

Blood haemoglobin was estimated on 108 willing athletes using cyan methaemoglobin method (Raghuramulu *et al.,* 2003). Procedure is appendedin (Appendix VI).

C. Clinical Examination

Clinical Examination provides an important added dimension to the overall assessment of the individual (Robert and William, 2000).

In the present study clinical examination was done with the help of a general physician by examining the athletes from head to foot for signs and symptoms using clinical assessment profoma using the classification of clinical signs and symptoms (Srilakshmi, 2010) and by asking the athletes if they felt any symptoms of nutritional inadequacy. Incidence of diseases in the families and morbidity among athletes (in the past 6 months) was interrogated and recorded.

D. Dietary survey

Dietary assessment is defined as the set of methods that measured and /estimate food intake which can be converted to nutrient intake by means of food consumption tables(Dwyer, 2000). In the present study, dietary intake was assessed using 24 hour food recall outlined by Kapil *et al.,* (2003). The intake of food and nutrients was compared using suggested food allowance by the ISLI, NIN and SAI (2007) for specific event and ICMR (2010).

Estimation of energy expenditure

The energy expenditure pattern was calculated using a physical activity questionnaire from which the total energy expenditure of the athletes was calculated (William, 2005). The energy cost of participation in an activity was calculated by time spent in work out multiplied by calorific value per minute (Appendix VII).

Determination of Fluid intake

Fluid intake was determined by distributing suitable standard bottle (1000 ml) for each athlete. The athletes were strictly instructed to pour whatever fluid consumed into the bottle and drink from the bottle. The quantity of fluid consumed by individual athletes was recorded for 7 days and the average fluid consumption per day was calculated. The quantity of fluid intake was compared with the fluid intake of 8l/day recommended by Venkataramana (2009).

E. Assessment of Physical Performance

Physical fitness is the ability to perform occupational, recreational, and daily activities without becoming unduly fatigued. Physical fitness component i.e cardio

respiratory endurance, speed, explosive strength, explosive power of leg muscles, muscular endurance, strength of abdominal muscles and agility were measured using the standard procedure adopted from the methodologies for Fitness Assessment (Ray *et al*., 2011) and American Alliance for Health and Physical Education Recreation and Dance(AAHPERD,1976) (Appendix VIII). (Plate 5a to 5d)

Plate 6 depicts warm-up exercise by Thang-Ta athletes and Plate 7 performance of Thang-Ta.

a. Vertical jump

c. Push ups

b. Standing broad jump

d. Queen college step test

Plate 5. Performance test

Plate 6.Thang –Ta Athletes Performing Warm- up Exercise

Plate 7.Athletes performing Thang -Ta

PHASE III

NUTRITION INTERVENTIONS AND IMPACT EVALUATION

A. Supplementation with *Hibiscus sabdariffa Linn* juice

1. Formulation and Standardisation

The fresh calyces of *Hibiscus sabdariffa Linn* (**(Plate 8)** were collected from the local market of Manipur during the month of November. The calyces were washed with clean water and then sundried for 5 days. Then the calyces were powdered in a mixer grinder to make into powder form. The investigator prepared the sports drink from *Hibiscus sabdariffa Linn* by different variation adding 1.5g, 2.5g, 3g of calyces powder in 240 ml of water (Plate 9) and multiple transportable carbohydrates (MTC) ie 2:1 proportion of glucose (14g): fructose (7g) and salt 0.08 g and 2:1 glucose (12): fructose (6). Multiple transportable carbohydrates enhance gastric emptying (Jeukendrup and Moseley, (2010), water absorption and fluid delivery (Summers *etal,*1995) and provide and provide superior endurance performance (Currel and Jeukendrup, 2007). The quantity of carbohydrate (glucose and fructose) and salt were added as per the recommended amount for ideal sports drinks (2:1:0.08g) in 240 ml of water.The quantity of carbohydrate (glucose and fructose) in the drink was 6-8%as per ideal recommendation.(American College of Sports Medicine et *al.,* 2007; American Dietetic Association *et al*., 2009).

Fresh calyces

Dried calyces

Plate 8. *Hibiscus sabdariffa Linn*

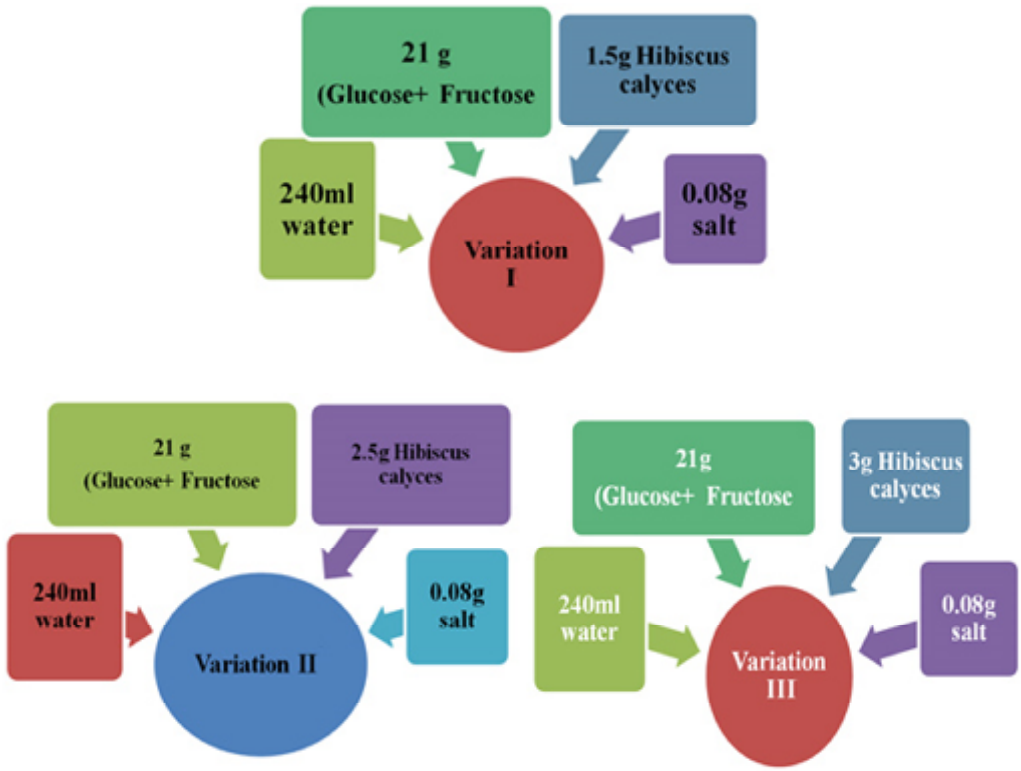


Plate 9. Different Variations of Hibiscus Drink

2. Acceptability Trials among Athletes

Sensory evaluation is a multidisciplinary science that uses human panelists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of the food products. Thus the quality of food is judged in terms of taste, color, consistency and flavor (Chandrasekhar, 2002)

The formulated drinks were provided for sensory evaluation to a semi trained panel of 10 students of physical education in Avinashilingam University and 18 judo athletes from training center Mayang Imphal and evaluated using the 9 point hedonic scale. It was observed that 2.5 g of *Hibiscus sabdariffa Linn* powder, 14 g glucose, 7g fructose, 0.08g salt with 240ml of water obtained the best score for acceptability and was named Hibisa drink and used as the supplement in the study. The acceptability scores are shown in Table I.

TABLE I

ACCEPTABILITY SCORE

Variations	Colour	Flavour	Taste	Consistency
I	5.44±0.91	4.89±1.10	3.96±0.96	5.68±0.80
II	8.10±0.10	6.60±0.99	6.71±0.89	6.57±0.79
III	8.80±0.40	5.24±1.23	4.16±1.04	5.68±0.80

Variation II (containing 2.5 g of sundried and powdered calyces of *Hibiscus sabdariffa Linn*, 14g of glucose, 7g of fructose and 0.08g of salt in 240 ml of portable water) obtained the highest scores for all the organoleptic parameters namely colour (8.10±0.10), flavor (6.60±0.99), taste (6.71±0.89) and consistency (6.57±0.79). Hence it was selected for supplementation to the athletes and given the name, Hibisa

3. Nutrient Analysis

Owing to financial constraints, selected components such as pH, totalsugar, total carbohydrate, sodium, potassium, iron, vitamin C and total antioxidant of (Variation II) Hibisa juice were analysed using standard procedures (Appendix IX).The value are presented in Table II.

TABLE II

COMPOSITION OF HIBISA DRINK

Components	Hibisa drink	
	Initial day	After 7 days
pH	3.58	2.96
Total sugar (g/dl)	33	30
Total carbohydrate (g/dl)	6	5
Sodium (mg/dl)	14	14
Potassium (mg/dl)	230	224.0
Iron (g/dl)	6.92	6.90
Vitamin C (mg/dl)	26	23
Total antioxidant (µg/ml)	216.8	198.6

It was observed that the formulated (Variation II) Hibisa drink was acidic with a pH of 3.58. Total sugar and total carbohydrate content were 33g/100g, 6g/100g respectively. Electrolytes such as sodium and potassium content were 14mg/100g, 230mg /100g respectively. Iron, vitamin C, and total antioxidant content were 6.92mg/100g, 26mg/100g, and 216.8 µg/ g respectively. After seven days of storage, the nutrient composition was slightly reduced. Acidity was increased with a pH of 2.96, total sugar, total carbohydrate, were reduced to 30g/100g, 5g/100g respectively. Sodium content was unchanged. Potassium content was reduced to 224.0 mg/100g. Iron content was almost same to 6.90 mg/100g. Vitamin C and total antioxidant content was reduced to 23mg/100g and 198 µg/g respectively. This observation shows that nutrient content of the drink was decrease slightly after storage of 7 days, acidity increased after storage of 7 days.

The pH of some of the commercially available sports drinks such as Gatorade Mandarine ,Gatorade Green Apple ,Gatorade Red Orange, Gatorade Artic snow, Gatorade Orange, Gatorade Citron were 3.3,3.2,3.2,3.4,3.0 and 3.1 respectively (Mettler *et al.,* 2006). This shows that the pH of Hibisa drinks 3.58 was comparable with the available pH of commercial sports drink. Iron content of the Hibisa drink was retained. For longer duration it is advisable to add pH regulator. Table III reveals the microbial count of the selected variation II of Hibisa on storage.

TABLE III

CHANGES IN MICROBIAL COUNT IN HIBISA DRINK

Hibisa drink	Storage
1 day	$7.5x10^{1}$
7 days	$6.3x10^{2}$
14days	$5.9x10^{4}$

The microbial count of Hibisa drink at different durations of storage showed that the viable count was in the acceptable range (10^{3} -10^{6}) (Gilbert et al., 2000).The procedure for microbial analysis is given in Appendix X.

4. Administration of *Hibiscus sabdariffa Linn* juice

The formulated Hibisa drink was manufactured on a large scale in the Likla Food Industry of Manipur for supplementation to the athletes. It was packed in 5400 (240 ml each) glass bottles, sterilized for microbial safety (Plate 10).

Plate 10.Preparation and Bottling of Hibisa Drink

No preservative was added. Freshly prepared bottles of Hibisa drink, (at the rate of 2 bottles per day/athlete) were rationed on a weekly basis, for 90 days of supplementation.

The athletes in the experimental group consumed the drink 10- 15 minutes before, during and after the practice. The same quantity of sweetened salted water (SSW) i.e plain water, glucose and fructose in the ratio of 2:1 and 0.08 g added salt was administered to the placebo group.

5. Evaluation of the Effect of Supplementation

Biochemical parameters namely hemoglobin, serum ferritin, blood glucose, lactate dehydrogenase, and Physical Fitness parameters namely, cardio respiratory endurance, speed, and explosive strength, explosive power of leg muscles, muscular endurance, strength of abdominal muscles and agility were assessed from base line for every 30 days (0 day, 30^{th} day, 60^{th} day), till final completion on 90^{th} day. The scores obtained were compared with the initial scores and the impact of supplementation was assessed.

B. Preparation of Nutrition Education Material and Imparting Nutrition Education

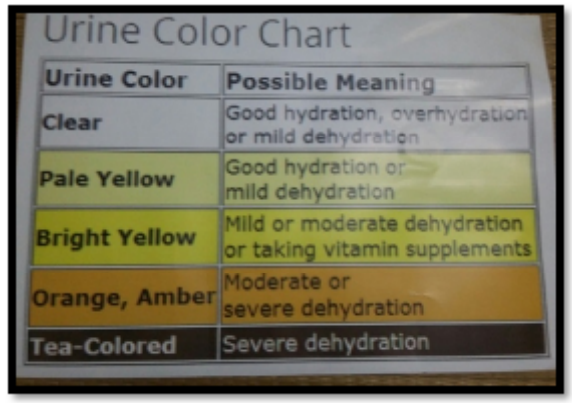
1. Formulation of Nutrition Education Materials

All the 364 athletes were included in the nutrition education programme. The nutrition education questionnaire formulated was administered to the athletes and evaluated to understand their initial nutrition knowledge. Based on their initial nutrition knowledge, education materials were prepared.

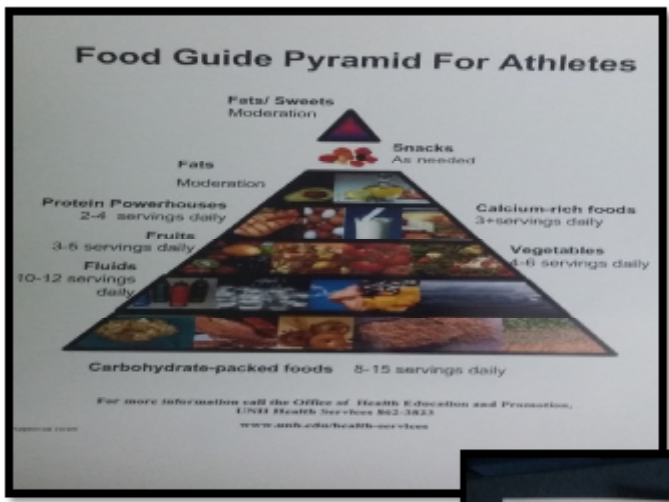
The investigator developed pamphlets Charts, Posters and a Power point presentation (Plate 11) on basic nutrition (facts), Sports nutrition, Hydration guidelines, Food pyramid etc for the Thang - Ta athletes by referring Nutrition and Hydration in Sports Guidelines for the Excellence, ILSI, NIN,SAI, 2007; (Duvillard *et al.,*2004; Welch ,2010; Mcardle,1996).

Power Point Presentation prepared by the researcher was projected to the athletes as it is a way of attracting audience towards expression of their views and arguments. Power point presentation combines audio and visual, making it easier for the audience to understand the concepts. Even the normal teaching or training becomes interactive by just using presentations in lectures (Paul, 2008). Nutrition education was imparted and the impact of education programme was evaluated using the same questionnaire.

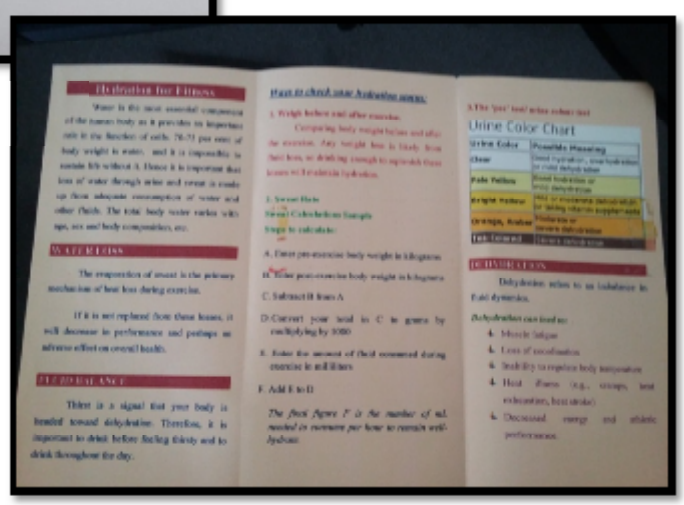
Powerpoint presentation



Urine color Chart



Food Pyramid



Pamphlet – Hydration guidelines

Plate 11. Education Materials

2. Imparting Nutrition Education

The investigator conducted the education program (Plate 12) in their respective training centers. Selected athletes from each district were given education program each day, for nine days at each center to complete the education program for all the selected athletes. Details on basic nutrition such as food groups, food pyramid, nutrient content of different foods, balanced diet, pre, during and post competition meals, importance of hydration, hydration guidelines, do's and don'ts for athletes were taught to the athletes using black board, charts, posters and power point. After the presentation, pamphlets, charts and posters and booklets were distributed, to make the nutrition education effective and helpful to the Thang-Ta athletes.

Plate 12. Imparting Nutrition Education

3. Evaluation of the Impact of Nutrition Education

The impact of nutrition education programme was evaluated by administering the same nutrition education questionnaire which was provided initially to all the athletes, again, and the improvement in nutrition knowledge, attitude and practice (KAP) was assessed by comparing the information given by the athletes in the proforma, before and after the nutrition education programme.

C. Statistical Analysis

The statistical analysis was done by using the SPSS software packages Version 17.0. The data were interpreted and analysed by using mean, standard deviation, ANOVA (Analysis of variance), ANACOVA (Analysis of co-variance), T test, F test and Z test. For repeated measures ANOVA, posthoc test namely Student Newman Kouls (SNK) test was conducted, if the ANOVA f-Test value was found significant .this is done to find which period differs significantly their mean value