

Study on the efficacy of supplementation of functional beverage on the blood profile of Sportswomen

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Abstract- Sports drinks are much relevant to the Indian sports scenario because of the fact that many of the Indian sports persons are undernourished and anaemic. Functional beverage was prepared by using whey water, pearl millet [*Pennisetum typhoideum*], cauliflower [*Brassica oleracea var. botrytis*] leaf powder, banana and jaggery at three different levels i.e. S₁, S₂ and S₃. The developed functional beverage was organoleptically evaluated by a panel of judges and students by using nine-point hedonic scale. Both the panels gave the highest overall acceptability scores to the S₁ level which was prepared by using 2.5 g cauliflower leaf powder, 5 g pearl millet, 10 g jaggery, 20 g banana and 63 ml whey water per 100 ml. The most acceptable level was chemically analyzed. Thirty sportswomen in the age group of 16 to 18 years were selected from Punjab Agricultural University, Ludhiana. The study was divided into two periods i.e. control and experimental. During control period the subjects were observed without supplementation for a month while during experimental period the subjects were supplemented with 200 ml of developed functional beverage for 3 months. The haematological profile, blood glucose and serum retinol level of the subjects were analyzed. Significant ($p \leq 0.01$) improvement was seen in haemoglobin, packed cell volume and mean corpuscular volume after the experimental period i.e. 9.42, 3.35 and 2.08 %, respectively. Seventy seven percent subjects were anaemic before the study which was reduced to fifty percent after 3 months. Significant ($p \leq 0.01$) improvement was also observed in blood glucose and serum retinol level i.e. 12.7 and 5.46 % in the subjects after the experimental period. Hence, it can be inferred from the results that supplementation of functional beverage before the sports training improved the blood profile of the sportswomen.

Index Terms- functional beverage, haematological profile, organoleptically, cauliflower [*Brassica oleracea var. botrytis*] leaf powder, whey water.

I. INTRODUCTION

In today's world of neck and neck competition, a sportsperson cannot afford to take chance in any area, as minute action can deprive him of fame and fortune. The ability to recover from all of these stresses can be the difference between a champion and a loser. The prerequisite for optimum levels in sports performance is not just good training but also a carefully maintained health.

Sports drinks are much relevant to the Indian sports scenario because of the fact that many of the Indian sports persons are undernourished and anaemic. Being a tropical country, Indian athletes tend to get exhausted quickly particularly during summer

and this affects their performance. A direct relationship has been found between haemoglobin and serum retinol concentration. Dietary deficiency of vitamin A and iron frequently coexist in developing countries and vitamin A deficiency appears to affect iron transport and RBC production (Lynch, 1997).

Some of the underutilized foods like whey water, cauliflower leaves, pearl millet, banana and jaggery can be utilized for the improvement of the nutritional status of sportswomen.

Whey is a nutritious by-product from cheese, *chhana* and *paneer* containing valuable nutrients like lactose, proteins, minerals and vitamins etc., which have indispensable value as human food (Parekh, 2006). In India, it is estimated that about 100 million kg of whey is annually derived as a by-product which may cause substantial loss of about 70,000 tonnes of nutritious whey solids. In addition, it is adding Biological Oxygen Demand (BOD) load to effluent (approx 35,000 to 45,000 mg / L) (Parekh, 2006).

In India, the consumption of green leafy vegetables is very low and is much below the recommended dietary allowances. Therefore, majority of Indians don't meet sufficient vitamins and minerals present in leafy vegetables. Cauliflower [*Brassica oleracea var. botrytis*] bears extensive leaves which are removed and discarded prior to cooking. The proportion of cauliflower and leaves arriving in the market is 2:1 of which the discarded leaves contribute to approximately 50 per cent of total production of cauliflower and dehydrated greens are considered concentrated sources of nutrients, addition of small amounts of these foods in various dietary preparations could be of immense value to combat the global prevalence of micronutrient malnutrition (Kowsalaya and Vidhaya, 2004).

In India, Pearl millet [*Pennisetum typhoideum*] is the fourth most important staple food crop after rice, wheat, and sorghum. The grain of pearl millet is particularly rich in iron and zinc and has high levels of fat and considered to have one of the best protein quality or amino acid score as compared to other cereals. Nutritional quality of pearl millet is improved by traditional processing practices viz. soaking, dehulling, fermentation, germination and malting. Thus, the pigments and anti-nutritional factors present in pearl millet could be reduced by using these simple home level processing methods (Archana et al 2000).

Bananas [*Musa paradisiaca*] are power house of nutrients. They originally come from Malaysia. Bananas and nutrition go hand in hand. They are great sources of an array of nutrients, with B-complex vitamins, vitamin C, potassium, manganese and magnesium. They are also important source of vitamin B (Cheamsawat, 2003) especially thiamine, which has an effect on fatigue reduction.

Jaggery [*Saccharum officinarum*] is a traditional unrefined non-centrifugal whole cane sugar consumed in Asia, Africa, Latin America, and the Caribbean. It is a concentrated product of cane juice without separation of the molasses and crystals.

Keeping in view the beneficial effect of underutilized foods the present study was planned to study the impact of supplementation of functional beverage on the haematological profile, blood glucose and serum retinol level of sportswomen.

II. MATERIALS AND METHODS

A. Development and sensory evaluation of functional beverage
Functional beverage was developed at three different levels i.e. S₁, S₂ and S₃ using whey water, pearl millet, cauliflower leaf powder, banana and jaggery. The organoleptic evaluation of developed functional beverage was done by the panel of judges using the Hedonic rating scale.

B. Chemical analysis of developed functional beverage
Most acceptable level of the developed functional beverage was chemically analyzed for proximate composition (AOAC, 1990), total soluble sugars (Dubois et al 1956), reducing sugars (Nelson, 1944), non reducing sugars (by calculation), iron (AOAC, 2000), calcium (AOAC, 1980), ascorbic acid (AOVC, 1996) and beta-carotene (Rao, 1967).

C. Selection and feeding of the subjects

Thirty sportswomen in the age group of 16 to 18 years were selected from Punjab Agricultural University, Ludhiana. General and sports information pertaining to age, religion, residential status, family size, menstruation, duration of menstruation was recorded for all the subjects through questionnaire schedule. The whole study was divided into two periods i.e. control period and experimental period. All the observations were recorded before the study, after one month of control period and after three months of experimental period. During control period the subjects were observed without the supplementation for a month while during experimental period the subjects were supplemented with 200 ml of developed functional beverage for a period of three months. The beverage was given half an hour before their sports activity.

D. Blood analysis of the selected subjects

Blood analysis was done for glucose (Glucometer), Haemoglobin (Dacie and Lewis, 1975), Packed Cell Volume (Raghuramula et al 2003), Mean Corpuscular Haemoglobin Concentration (MCHC) (Raghuramula et al 2003), Mean Corpuscular Volume (MCV) (Dacie and Lewis, 1975), Total Iron Binding Capacity (Teitz, 1976) and Serum Retinol Level (Raghuramulu et al 2003).

E. Statistical analysis

The data on all the blood parameters was analyzed statistically. The mean standard error, percentages, paired t- test and their statistical significance was ascertained using a computer programme package (Cheema and Singh, 1990).

III. RESULTS AND DISCUSSIONS

The study was conducted on 30 sportswomen between the age group of 16 to 18 years studying in Punjab Agricultural University. Majority of subjects 67 percent were Sikh and rest 33 percent were Hindu. Most of the subjects were hostellers (73

percent) while 27 percent were day scholars. Majority of subjects belong to medium family with family size of 4-6 members. The menstrual flow was normal in 77 percent of the subjects and rest 23 percent was having heavy flow (Table 1).

Table 1: General Information of the subjects

Characteristics	Total (N=30)
Age (years):	
16 -18	30 (100)
Religion:	
Hindu	10 (33)
Sikh	20 (67)
Residential status:	
Hostellers	22 (73)
Day Scholars	8 (27)
Family size:	
Small (< 4)	1 (3)
Medium (4- 6)	28 (93)
Large (>6)	1 (3)
Menstruation:	
Normal	23(77)
Heavy	7(23)
Duration, Days:	
2-3	8 (27)
4-5	18 (60)
>5	4 (13)

Figures in parenthesis are percentages

The developed functional beverage was organoleptically evaluated by a panel of judges from the department of food and nutrition, Punjab Agricultural University, Ludhiana and students by using nine-point hedonic scale to judge the acceptability of the product and the scores given by the trained panel for color, appearance, flavor, texture, taste as well as overall acceptability of the developed functional beverage varied from 7.18 to 7.7, 6.7 to 7.70, 6.4 to 7.5, 6.2 to 7.4, 6 to 7.8 and 6.3 to 7.8, respectively. The corresponding scores given by the student's panel varied from 7.5 to 7.7, 6.8 to 7.3, 6.3 to 7.5, 5.8 to 7.4, 6.5 to 7.7 and 6.2 to 7.8, respectively. According to the both panels the most acceptable level of beverage was having 2.5 g cauliflower leaf powder, 5 g pearl millet, 10 g jaggery, 20 g banana and 63 ml whey. It had the overall acceptability score of 7.8±0.78 (Table 2).

Hundred grams of developed functional was analyzed for proximate composition showed that it had 81 g of moisture, 2.8 g of crude protein, 0.4 g of crude fat, 0.9 g of crude fibre, 0.85 g of ash, 14.05 g of carbohydrates and provided 71 Kcal of energy. Developed functional beverage had 11.8 g total soluble sugars,

4.55 g of reducing sugars, 7.25 g of non-reducing sugars and 4.6 g of starch. The concentration of minerals iron and calcium in Table 2: Organoleptic evaluation of developed functional beverage

S. No	Colour	Apperance	Flavour	Texture	Taste	Overall acceptability
Trained Panel						
S 1	7.7±0.58	7.70±0.66	7.5±0.79	7.4±0.78	7.8±0.43	7.8±0.78
S 2	7.63±0.48	7.6±0.48	7.3±0.46	7.1±0.33	7.1±0.48	7.3±0.43
S 3	7.18±0.63	6.7±0.43	6.4±0.69	6.2±0.63	6±0.5	6.3±0.43
F-Ratio	3.4*	17.9*	12.4*	16.8*	58.5*	23.1*
C.D. at 5%	0.42	0.38	0.49	0.45	0.34	0.43
Students Panel						
S 1	7.5±0.47	7.3±0.47	7.5±0.5	7.4±0.5	7.7±0.47	7.8±0.37
S 2	7.7±0.47	7.3±0.47	7±0	7±0	7.2±0.37	7.2±0.37
S 3	7.5±0.5	6.8±0.37	6.3±0.74	5.8±0.37	6.5±0.5	6.2±0.37
F-ratio	0.20 ^{NS}	2.14 ^{NS}	6.4*	23.2*	8.41*	25.3*
C.D. at 5%			0.69	0.49	0.61	0.5

S 1= functional beverage with 2.5 g cauliflower leaf powder, 5 g pearl millet, 10 g jaggery, 20 g banana and rest whey water to raise the volume to 100ml

S 2 =functional beverage with 3 g cauliflower leaf powder and rest ingredients same as in S1

S 3 =functional beverage with 3.5 g cauliflower leaf powder and rest ingredients same as in S1

Values are Mean ± S. E

*: Significant at 5% level.

functional beverage was 5.51 mg and 103 mg, respectively. The concentration of vitamins, ascorbic acid and β-carotene was 2.7 mg and 1185 µg, respectively.

At the end of three months with supplementation of functional beverage a significant ($p \leq 0.01$) increase was observed in the haemoglobin level (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), blood glucose and serum retinol level of the subjects (Table 3 and 4).

The mean haemoglobin (Hb) of the subjects before and after the control period was 10.73 and 10.77 g/dl and after the three months of experimental period the corresponding value was 11.89 g/dl. The Hb of the subjects was less than the reference Hb value of 12 g/dl (WHO, 2001). The poor iron status of the sportswomen indicated by their low haemoglobin levels could be associated with iron losses from gastrointestinal bleeding, consumption of pain relievers, blood loss in urine from damaged muscles, excessive sweat and excessive exercise. Eichner (1992) and Kim et al (2002) reported that sportswomen are especially susceptible to poor iron status because of the blood loss experienced during menstruation but the primary cause is the inadequate iron in their diet. However, Portal et al (2003) reported that the most common cause of the decrease in athlete's haemoglobin is pseudo-anaemia which resulted from an increase in plasma volume during a sporting performance.

The value of mean packed cell volume (PCV) of the subjects before the study was 31.1%. The corresponding values after control and after experimental period were 31.16 and 32.24%. The reference PCV range is 37-44% (Dacie and Lewis, 1975). The corresponding value of PCV obtained was below the normal range indicating anaemic condition of the subjects. However, the statistical analysis revealed a significant ($p \leq 0.01$) increase in PCV after the supplementation period. Kaur (2002) reported the PCV range of the sportswomen to be 24.2 to 42.6%. Malkit (1987) reported a range of 33.9 to 35.0% of PCV of the girls, whereas the PCV of Hockey and Handball players as observed

by Kaur (1988) was 36.9% and 44.8% before and after the exercise response, respectively.

The initial mean corpuscular volume (MCV) value of the sportswomen was 90.04 fl. The corresponding values before and after the three month experimental period were 90.1 and 92.01 fl, respectively. The normal MCV range is 77-95 fl (Dacie and Lewis, 1975). The corresponding value of MCV before and after the study was in-between the normal range. A significant ($p \leq 0.01$) increase was seen in the MCV value of the subjects after the supplementation period.

The initial mean corpuscular haemoglobin concentration (MCHC) value of the subjects was 32.63% and the corresponding values before and after the experimental period were 32.44 and 33.01%, respectively. The normal MCHC range is 30-33% (Dacie and Lewis, 1975). The corresponding value of MCHC after the supplementation was slightly high than the normal range but there was a non-significant ($p \leq 0.01$) difference seen in the MCHC value of the subjects after the supplementation period. Kaur (1988) reported a range of 17.1 to 48.9% with a mean value of 26.8±0.1 and 29.1±1.6% before and after the exercise in hockey and handball players. Kaur (2002) reported the MCHC range of the sportswomen which varied from 25.4-46.6%.

The initial mean total iron binding capacity (TIBC) value of the subjects was 236.3 µg/dl. The TIBC of the subjects before and after the supplementation period was 237.5 and 235.8 µg/dl, respectively. A non significant ($p \leq 0.01$) change was seen in the TIBC of the subjects after the supplementation period. It was observed that the TIBC value was lower than the normal range. The normal TIBC range is 250-416 µg/dl (Goodhart and Shills, 1990). Bains (1995) reported that the mean value of TIBC for young girls was 429 µg/dl. Onno et al (1998) revealed that TIBC, which is a measure of both unsaturated and saturated transferrin, is already affected before the iron supply fails: TIBC rises as storage iron decreases. Santolo et al (2008) evaluated the effects

Table 3: Hematological profile of the subjects before and after supplementation of functional beverage by the subjects

Blood parameters	Control period		Experimental period After 3 months	% Change		Normal values	t-value		
	0 month	After 1 month		(a vs b)	(b vs c)		a vs b	b vs c	c vs a
	a	b		(a vs b) (b vs c)					
Haemoglobin, g/dl	10.73 ± 1.07	10.77 ± 1.04	11.89 ± 0.82	0.37	9.42	> 12a	1.42 ^{NS}	7.9**	7.47**
PCV, %	31.14 ± 6.61	31.16 ± 6.61	32.24 ± 2.56	-	3.35	37 – 44b	0.11 ^{NS}	6.86**	7.74**
MCV, fl	90.04 ± 0.43	90.1 ± 0.33	92.01 ± 1.51	0.07	2.08	77 – 95b	1.45 ^{NS}	3.86**	4.11**
MCHC, %	32.63 ± 1.8	32.44 ± 2.02	33.01 ± 1.36	-	1.73	30 – 33b	1.18 ^{NS}	1.2 ^{NS}	0.86 ^{NS}
TIBC, µg/dl	236.3 ± 32.9	237.5 ± 32.9	235.8 ± 20.59	0.5	0.7	250 – 416c	1.25 ^{NS}	1.31 ^{NS}	1.23 ^{NS}

** : Significant at 1 % level NS : Non-Significant

a : WHO (2001), b: Dacie and Lewis (1975), c : Goodhart and Shills (1990)

of regular physical exercise on anemia and iron status as it reduces serum iron and transferrin saturation, and elevates soluble transferrin receptors young non-professional female athletes. They observed that physical exercise has an impact on iron. Nearly one fifth of recreational athletes have anemia and a third have iron deficit, these conditions can decrease their physical performance.

The mean glucose level of the subjects before the study was 120.6 mg/dl. The corresponding values before and after the supplementation period were 121.4 and 139 mg/dl, respectively (Table 4). The obtained value was in-between the normal range given by Raghuram et al (2007). There was a significant ($p \leq 0.01$) increase in the blood glucose level after the supplementation period. Kanabur and Devi (2005) also reported that a sports drink

has a positive effect on the blood glucose level as compared to placebo.

The mean serum retinol level of the subjects before and after the control period was 24.67 and 24.93 µg/dl, respectively. After the three months supplementation period the corresponding value was 26.37 µg/dl. The normal value for serum retinol level is 25 µg/dl (Table 4). The increase could be due to the consumption of cauliflower leaf powder in the form of developed functional beverage. There was a significant ($p \leq 0.01$) increase in the serum retinol level after the supplementation period. A study conducted by Jood et al (2001) revealed that after the supplementation of 100 g cauliflower leaf powder per day to the school children the serum retinol level increased by 33.33%.

Table 4: Glucose and Serum retinol level of the subjects before and after the supplementation of functional beverage

Blood parameters	Control period		Experimental period After 3 months	% Change		Normal values	t-value		
	0 month	After 1 month		(a vs b)	(b vs c)		a vs b	b vs c	c vs a
	a	b		(a vs b) (b vs c)					
Glucose, (mg/dl)	120.6 ± 10.21	121.4 ± 9.85	139.0 ± 8.80	-	12.7	<200 [#]	1.63 ^{NS}	16.43**	12.56**
Serum retinol, (µg/dl)	24.67 ± 1.69	24.93 ± 1.70	26.37 ± 1.84	1.04	5.46	25 µg/dl [^]	1.43 ^{NS}	6.9**	7.1**

* : Significant at 5% level ** : Significant at 1% level

NS: Non-significant, #: Raghuram et al (2007), ^ : WHO (1998)

IV. CONCLUSION

The investigation of present study revealed that supplementation of 200 ml developed functional beverage prepared by using 5 g cauliflower leaf powder, 10 g pearl millet, 20 g jaggery, 40 g banana and 126 ml whey helped in improving the hematological profile, serum retinol level and blood glucose level of the subjects. This was reflected by significant ($p \leq 0.01$) increase in haemoglobin (9.42%), mean corpuscular volume (2.08 %), packed cell volume (3.35 %), mean corpuscular haemoglobin concentration (1.73%), blood glucose level (12.7 %) and serum retinol level (5.46 %) respectively. Hence it can be inferred from the results that supplementation of developed functional beverage significantly improved the blood profile of the sportswomen.

REFERENCES

- [1] AOAC (1980) Official Methods of Analysis 11th ed. Association of Official Analytical Chemists. Washington, DC.
- [2] AOAC (1990) Official Methods of Analysis 14th ed. Association of Official Analytical Chemists. Washington, DC.
- [3] AOAC (2000) Official Methods of Analysis 17th ed. Association of Official Analytical Chemists. Washington, DC.
- [4] AOVC (1996) Methods of Vitamin assay. Association of Vitamin Chemists Inc. (Ed.) Interscience Publishers. pp. 306-312.
- [5] Archana, Sehgal S and Kawatra A (2000) In vitro protein and starch digestibility of pearl millet as affected by processing techniques. Die Nahrung 45 (1):25-27.
- [6] Bains K K (1995) Impact of energy and iron status on the physical fitness among college girls. Ph.D. Dissertation, Punjab Agricultural University, Ludhiana.
- [7] Cheamsawat N (2003) Production process of syrup from banana and the by-products. Outstanding Researches 3:36.

- [8] Cheema H S and Singh B (1990) CPCSI- A computer program package for the analysis of commonly used experimental designs. Punjab Agricultural University, Ludhiana, India.
- [9] Dacie J V and Lewis S M (1975) Practical Haematology. 5th ed. English Language Book Society and Churchill Ltd., London, pp.32.
- [10] Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F (1956) Colorimetric methods for determination of sugars and related substances. Anal. Chem.28: 350.
- [11] Eichner E R (1992) Sports anaemia, iron supplementation and blood doping. Med. Sci. Sports Exerc.24: 315-18.
- [12] Goodhart R S and Shills M E (1980) Modern nutrition in health and disease. 6th ed. Lea and Febiger, Philadelphia Co., p. 334.
- [13] Jood S, Gupta M, Yadav S K and Khetarpaul N (2001) Effect of supplementation on haemoglobin and serum retinol levels and nutritional status of school children of northern India. Nutr. Health 15(2):97-111.
- [14] Kanabur V V and Devi K (2005) Development, nutritional analysis and evaluation of some sports drinks. J. Fd. Sci. Technol. 42: 415-18.
- [15] Kaur A (2002) Impact of haematinic supplementation to enhance the physical performance of sportswomen. M.Sc. Thesis. Punjab Agricultural University, Ludhiana, India.
- [16] Kaur N (1988) Assessment of nutritional status of girl athletes. Ph.D. Dissertation, Punjab Agricultural University, Ludhiana.
- [17] Kim S H, Kim H Y P, Kim W K and Park O J (2002) Nutritional status, iron-deficiency-related indices, and immunity of female athletes. The Intr. J. of Applied and Basic sci. Nutritional Sci. 18: 86-90.
- [18] Kowsalaya S and Vidhaya M R (2004) Nutritive value of selected dehydrated green leafy vegetables. Ind J. Nutr. Dietet. 41: 279-86.
- [19] Lynch S R (1997) Interaction of iron with other nutrients. Nutr Rev 55:102.
- [20] Malkit (1987) Assessment of nutritional status of young Punjabi women. Ph.D. Dissertation, Punjab Agricultural University, Ludhiana.
- [21] Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J. Bio. Chem. 153: 375-380.
- [22] Onno W V A, Basil T D, Virgil F F, Elaine W G and David A N (1988) Determination of serum iron, total iron binding capacity and percent transferring saturation, approved standard. Clinical and laboratory standards institute. NCCL, Wayne, Pennsylvania.
- [23] Parekh J V (2006) Emerging new technologies in the dairy industry in India. (Cited from <http://www.fnbnews.com/article/detarchive.asp?articleid=19393&ionid=40>).
- [24] Portal, Epstein S M and Dubnov G (2003) Iron deficiency and anemia female athletes cause and risks. Centre of sports medicine science and research, Wingate Institute, Jerusalem, Israel, 149 (10): 698-03.
- [25] Raghuram T C, Pasricha S and Sharma R D (2007) Diet and diabetes. ICMR, Hyderabad.
- [26] Raghuramula N, Nair K M and Kalayanasundram S (2003) *A manual of Laboratory techniques*. National Institute of Nutrition, ICMR, Hyderabad, India. pp. 308-09.
- [27] Rao C N (1967) True vitamin A value of some vegetables. Ind. J. Nutr. Dietet. 4:10-16.
- [28] Santolo M, Stel G, Banfi G, Gonano F and Cauci S (2008) Anemia and iron status in young fertile non-professional female athletes. Eur J Appl Physiol;102(6):703-9.
- [29] Teitz N W (1976) *Fundamentals of Clinical Chemistry*. W.B. Saunders and Co. p. 928.
- [30] WHO (1998) Immunization-linked vit A supplementation study group. Randomized trial to assess benefit and safety of vit A supplementation linked to immunization in early infancy. Lancet 352:1257-63.
- [31] WHO (2001) Iron deficiency anemia: Assessment, Prevention and Control. A guide for program manager. P-3. World Health Organization, Geneva.

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