Original Article

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Nutrition Section

Effect of Phytonutrient Rich Juice Blends on Antioxidant Status and Lipid Profile in Young Adults: A Randomised Trial

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ABSTRACT

Introduction: Phytonutrients like polyphenols, carotenoids, glucosinolates and phytoestrogens may not be as essential as micronutrients. But, these have been useful to prevent disease and keep the fitness of the body. Amla (Indian Gooseberry), guava, kokum and purple grapes are highly nutritious seasonal fruits, rich in these phytonutrients and micronutrients. Regular consumption of these fruit-based beverages can improve the antioxidant status and health of the young adults.

Aim: The aim was to study efficacy of two formulations (F1 and F2) as health beverages made using juices of amla and dark grapes as main ingredients to be assessed as antioxidant rich natural fortificants.

Materials and Methods: Fourty-eight healthy volunteers were recruited (age 18-35 years) and randomised in a double blind trial of four weeks comprising of three groups (placebo, F1 and F2). Placebo was pineapple flavoured sugar syrup, F1 was amla based syrup containing water extracts of tulsi, brahmi, bael and jambhul juice while F2 had purple grape juice as major ingredient along with guava, pomegranate and kokum juices in sugar syrup. Results were assessed using Two-way ANOVA followed by paired t-test and p-value <0.05 was considered significant.

Results: F1 resulted in significant decrease in total cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) levels (p-value <0.001, 0.001 and 0.01, respectively); while F2 resulted in significant lowering in plasma glucose, total cholesterol and HDL levels (p-value <0.05, 0.001 and 0.001, respectively). Both F1 and F2, showed a significant decrease in alkaline phosphatase. Further, F2 resulted in a significant increase of haemoglobin percentage and F1 exhibited decrease in SGOT. There was a significant effect of F1 and F2 on plasma MDA (biomarker of lipid peroxidation), Trolox Equivalent Antioxidant Capacity (TEAC), Catalase (CAT) and Super Oxide Dismutase (SOD). Two way ANOVA indicated time by treatment interaction terms between F1 and placebo to be significant for MDA, TEAC and SOD. In case of F2, differences between placebo and F2 were significant for Reduced Glutathion (GSH), and time by treatment interaction terms between F2 and placebo were significant for MDA and TEAC.

Conclusion: The present formulations as functional food products have shown a favourable effect on lipid profile and antioxidant status of healthy human subjects. These will have value as health beverages both at national and international level.

Keywords: Antioxidants, Bioefficacy, Peroxidation, Triglycerides

INTRODUCTION

Plant foods, in addition to their nutritional contents, are rich in large number of phytochemicals. In recent years, these have been termed as phytonutrients which may not be as essential as micronutrients but these have shown to prevent disease and help to keep the fitness of body [1]. Various polyphenols, carotenoids, glucosinolates and phytoestrogens are some examples of phytonutrients. In addition to fiber and micronutrients which contribute to the health effects, phytonutrients and antioxidants like polyphenols, are increasingly regarded as effective protective agents against ageing [1]. Considering the fact that chronic diseases like diabetes and Coronary Heart Disease (CHD) are the major challenges of this century, consuming phytonutrient rich fruits and vegetables could contribute both as therapeutic and the preventive approaches for these diseases [2]. Studies concerning the bioefficacy of antioxidants like ascorbic acid and polyphenols from natural fortificants are limited [3-7]. Better knowledge of bioefficacy is essential for investigating the health effects of polyphenols [8].

Polyphenols are abundant in berry fruits and polyphenol rich extracts of several berries have been shown to inhibit α-amylase and α-glucosidase activities [9]. As compared to a large number of in vitro studies, only limited reports are available for human studies [10]. Again, a juice using single source of polyphenols and other phytonutrients is likely to be less effective than the combination of different juices due to synergistic effects. Based on large number of edible plant materials screened for the antioxidant activity [11], using in vitro assays, two formulations were made (F1 and F2). It was aimed to study the efficacy of these two experimental phytonutrient enriched formulations and compare with placebo for antioxidant status and health status.

The objectives of the study were to study effect of formulations on blood antioxidant status (TEAC, plasma MDA, antioxiant enzymes) and lipid profile, haemoglobin, fasting glucose levels.

MATERIALS AND METHODS

A phase one type clinical trial was undertaken during October 2013-November 2013 at SWA and ARI hostels, Pune, Maharashtra, India, wherein healthy subjects aged 18-35 year participated. Adults with a normal complete blood count, LDL cholesterol >100 mg/dL, normal liver and renal function (normal levels of transaminases and alkaline phosphatase) were included in the study. Two senior team members of the research team personally visited and explained the work plan to the enrolled students. Informed written consent was taken from each of the subjects for their participation in the study from the institute where the study was conducted. Study was approved by the Institutional Ethics Committee of Bharti Vidyapith and also has been registered as clinical trial at CTRI (CTRI/2015/01/005402).

Exclusion criteria: Intake of pharmaceuticals and nutritional supplements, allergy against certain foods, pregnancy, lactation, presence of chronic diseases like diabetes, presence of diarrhea/

antibiotic treatment during experiment, high or low BP, Smoking and drinking habits.

Sample size of 45 with 15 in each group had been computed for an α of 0.05 (p-value) and power of 0.9. However, to even out the male to female ratio, eight male and eight females were included in each group.

At the screening visit, the participant's health status and medical history were assessed by using a structured interview along with anthropometric measurements. Fourty-eight healthy volunteers were recruited (age 18-35 years) and randomised in the double blind trial. This human trial was conducted comprising of three groups (16 subjects /group) F1, F2 and placebo [Table/Fig-1a,b].

Supplement	Carbohydrates (gm)	Fats (gm)	Proteins (gm)	Energy (Kcals)
Placebo (C)	20	0	0	80
F1	19.8±1.2	0.2±0.03	0.5±0.04	83±5
F2	18.5±0.9	0.3±0.02	0.6±0.02	83±3

[Table/Fig-1a]: Macronutrient composition per 100 gm.
Values represent mean±S.D

Supplement	Vitamin C (mg)	Iron (mg)	Total poly phenols (mg)	TE AC IC50	DPPH scavenging activity IC50
Placebo (C)	0	0	0	0	0
F1	116.2±12.8	1.06±0.04	157.6±7.8	6.45±0.14	975.9±140.1
F2	15.9±7.8	0.65±0.07	18.42±0.1	16.58±0.02	28.37±3.7

[Table/Fig-1b]: Micronutrient and antioxidant composition per 100 gm.
Values represent mean±S.D
Placebo (C): Sugar 100 gm, pineapple essence (Flying Bird 0.5 gm), edible yellow colour (0.5 gm), filtered water 889 gm. Method: Add 100 mL water to 100 gm sugar and make syrup by boiling, cool and add flavour and colour and store in cool place. Dilute four times to serve.
F1: Raw avla juice (80 mL), water extract of powders of tulsi, brahmi and bel leaves (10 mL) jambhul juice (10 mL, sarvottum, ratnagiri) and sugar 100 gm. Method: Add 100 mL water to 100 gm sugar and make syrup by boiling, cool and add 100 mL juice mixture and store in cool place. Dilute four times to serve.
F2: Purple grapes (70%), guava (10%), pomgranate (10%), kokum (10%). Method: Add 100 mL water to 100 g sugar and make syrup by boiling, cool and add juice mixture and store in cool place. Dilute four times to serve

F1 consisted of six different sources of antioxidants i.e., raw gooseberry juice (*Emblica officinalis*), Jamun juice (*Syzygium cumini)* and water extracts of powders of bael fruit, bael leaf (*Aegle marmelos*), Holy basil leaf (*Ocimum sanctum*) and Indian pennywort leaf (*Bacopa monnieri*). F2 comprised of juice blend of black grapes (*Vitis vinifera*) as main ingredient, pomegranate (*Punica granatum*), grape fruit (*Citrus paradise*), guava (*Psidium guajava*) and kokum (*Garcinia indica*). Basis for the formulations was the in vitro experiments done to decide the formulation by altering percentage of ingredient juices and our previous work on amla and grapes [12,13].

Study participants were served with beverages as placebo (100 gm drink with artificial fruit flavours and colours) or F1 or F2 served as fruit juices (100 gm, as drink using whole fruit purees) [Table/Fig-1a,b]. Fruits were procured from the local market and stored for the entire period of experiment at -20°C in order to avoid variation in their composition. During four weeks run-in period, the volunteers consumed the supplements every day in the evening before their dinner time. Daily attendance record was maintained throughout the study period. Subjects were asked to maintain their diary of routine activity and diet pattern throughout experimental period and requested not to consume unhygienic food from street vendors.

Analyses

At the start and end of the study period, fasting blood samples (10 mL) were collected after an overnight fast of 12 hours. Blood samples were analysed for lipid profile, haemogram, fasting glucose levels, haemoglobin, SGOT, SGPT and antioxidant status (plasma MDA, GSH, Cat, SOD, GPx, TEAC).

Plasma was separated by centrifugation for 20 minutes at 4°C and 3000 rpm. All analyses were carried out in the Biochemistry Laboratory at BVDU and RGITBT. Plasma glucose, total cholesterol and triglycerides, HDL, SGPT, SGOT were analysed on the ERBA Chem 5 autoanalyser (Transasia Bio Medicals Ltd. Mumbai, India) using Transasia kits. LDL cholesterol concentrations were calculated by using the Friedewald equation. HDL cholesterol concentrations were analysed by using the direct HDL cholesterol assay. Plasma MDA, GSH, Catalase, SOD, GPx, TEAC were measured with the use of the spectrophotometric assays (Schimadzu, Japan and Bio tek Eon, U.S) as reported in our previous studies [14].

The Coefficients of Variability (CVs) for inter- and intra-assay of glucose, total cholesterol, HDL and triglyceride values were <4%. CVs of inter- and intra assay for antioxidant markers were <5%.

STATISTICAL ANALYSIS

Data were expressed as means±SD. Statistical analyses were conducted with the use of SPSS Version 10.0.1. Descriptive statistics were computed to characterize the men and women. Data were also analysed for main effects of fortification and interactions using a Two-way ANOVA followed by paired t-test, p-value <0.05 was considered significant.

RESULTS

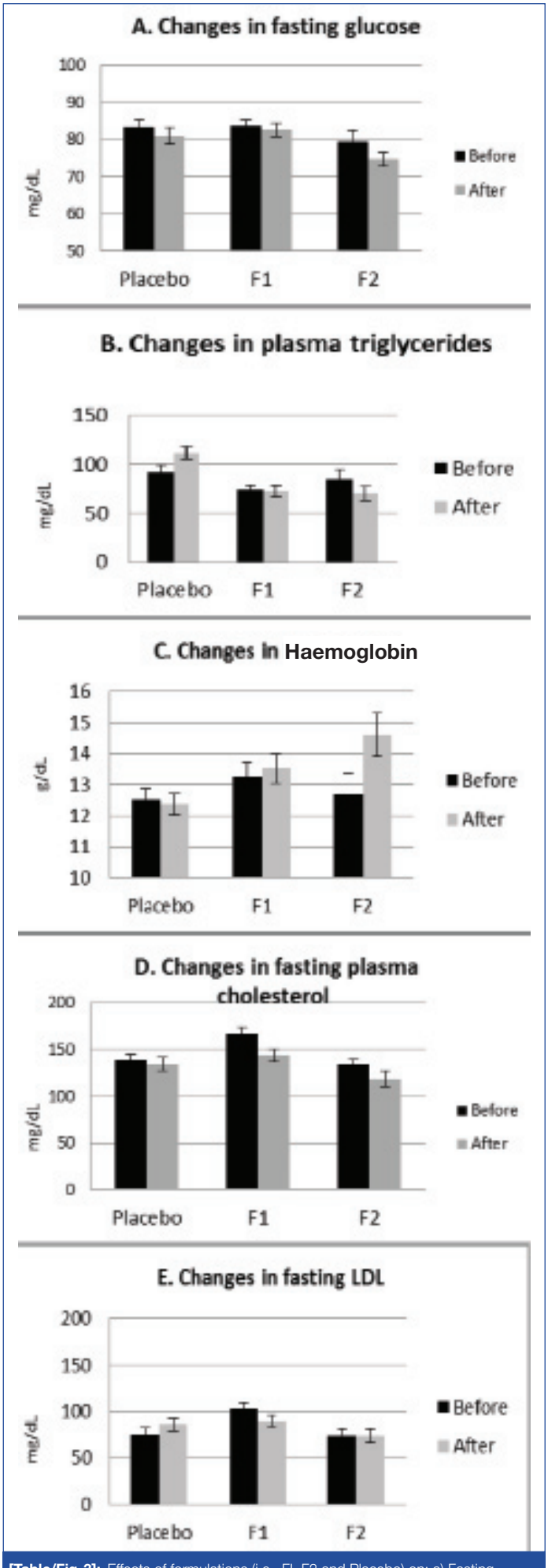
This was a phase one study, exploratory in nature. The basic characteristics of the participants enrolled in the three groups are presented in [Table/Fig-2].

Compliance was high and body weights were unchanged during the trial. The subjects in all the groups (Placebo F1 and F2) were matched for age, sex, ethnicity, and body mass index. Baseline subject characteristics and baseline lipid profiles are reported in [Table/Fig-2]. No significant differences in the baseline fasting glucose, lipid profile were observed between the three groups (p=0.2) [Table/Fig-3] indicates the changes in plasma glucose, lipid profile [Table/Fig-4-7] represents the results of effects over time for placebo, F1 and F2 as indicated through the paired t-test. In case of placebo group, the changes in the plasma glucose, lipid profile were not significant (p>0.10). F1 supplementation resulted in significant changes in total cholesterol, HDL and LDL levels (p-value <0.001, 0.001, 0.01, respectively). F2 resulted in significant changes in plasma glucose, total cholesterol and HDL levels (p-value <0.05, 0.001, 0.001, respectively).

[Table/Fig-8] indicates the changes in SGOT, SGPT. [Table/Fig-5]

Characteristics	Placebo (men)	Placebo (women)	F1 (men)	F1 (women)	F2 (men)	F2 (women)
Age (years)	26±8	28±7	27±5	26±6	28±5	28±6
BMI	18.2±2.1	17.9±3.0	22.3±3.2	23.6±4.6	23.1±2.4	23.6±0.5
Plasma glucose (mg/dL)	86.6±6.1	81.5±7.7	84.1±4.3	83.6±8.4	80.7±9.8	81.6±8.6
Serum total cholestrol (mg/dL)	126.8±25.1	145.6±26.7	166.5±27	166.8±30.8	137.7±20.1	138.6±16.3
Serum LDL cholestrol (mg/dL)	73.5±22.4	73.9±33.9	111±26	95.9±19.6	79.9±18.7	69.2±24.1
Serum HDL cholestrol (mg/dL)	40.6±7.9	52.8±12.6	40.5±5.7	56.2±15.2	45.1±13.1	50.2±4.4
Serum TG (mg/dL)	101.8±11.8	89.7±12.8	74.9±28.9	72.9±12.3	63.7±12.1	95.5±30.4

[Table/Fig-2]: Characteristics of participants.
Values represent mean±S.D



[Table/Fig-3]: Effects of formulations (i.e., FI, F2 and Placebo) on: a) Fasting glucose; b) Plasma triglycerides; c) Haemoglobin; d) Fasting plasma cholesterol; e) Fasting LDL.

Formulation	Value	Glucose mg/dL	TG mg/dL	CHOL mg/dL	HDL mg/dL	VLDL mg/dL	LDL mg/dL
Placebo	t-value	1.20	5.67	1.13	4.06	5.67	–2.32
	p-value	>0.2	<0.05	>0.2	<0.01	0.10	0.10
F1	t-value	1.35	0.13	4.20	5.43	0.13	3.04
	p-value	0.20	0.89	0.001	0.001	0.89	0.01
F2	t-value	2.04	0.52	3.41	5.10	0.30	1.09
	p-value	0.05	0.26	0.001	0.001	0.34	0.10

[Table/Fig-4]: Analysis of changes in fasting glucose and lipid profile.
TG: Triglycerides; CHOL: cholesterol: HDL: High Density lipoprotein; VLDL: Very Low Density lipoprotein; LDL: Low Density lipoprotein.

Formulation	Value	SGOT u/dL	SGPT u/dL	ALP u/dL	HB gm%
Placebo	t-value	1.97	0.61	NA	–1.15
	p-value	0.10	>0.2	NA	>0.2
F1	t-value	2.22	0.73	4.53	–0.42
	p-value	0.04	0.48	0.00	0.68
F2	t-value	–0.69	–1.08	2.12	–2.33
	p-value	0.35	0.35	0.07	0.01

[Table/Fig-5]: Analysis of changes in liver enzymes and haemoglobin.
SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic transaminase; ALP: Alakaline phophatase; Hb: haemoglobin.

Source of variation	Biomarker	F	p-value
Supplement	GSH	0.193721	0.665725
Time (days)		90.64717	5.41E-08
Interaction		0.000537	0.981805
Source of Variation		**F**	**p-value**
Supplement	MDA	0.145514	0.705102
Time (days)		15.31215	0.000388
Interaction		8.649237	0.005688
Source of Variation		**F**	**p-value**
Supplement	SOD	0.840417	0.36351
Time (days)		30.67893	1.02E-06
Interaction		5.733561	0.020283
Source of Variation		**F**	**p-value**
Supplement	TEAC	1.078272	0.303547
Time (days)		5.1579	0.027005
Interaction		3.277722	0.075592

[Table/Fig-6]: ANOVA for Comparison of placebo vs F1
GSH: reduced glutathione; MDA: malonedialdehyd; SOD: Super Oxide Dismutase; TEAC: Trolox Equivalent Antioxidant Capacity

gives the comparison of liver enzymes and haemoglobin before and after supplementation. The values for SGOT, SGPT, Alakaline Phophatase (ALP) and Haemoglobin (Hb) remained unchanged in case of placebo group. But, both F1 and F2 showed significant decrease in ALP, F2 resulted in significant increase of Hb% (p-value <0.01) and F1 exhibited decrease in SGOT (p=0.043).

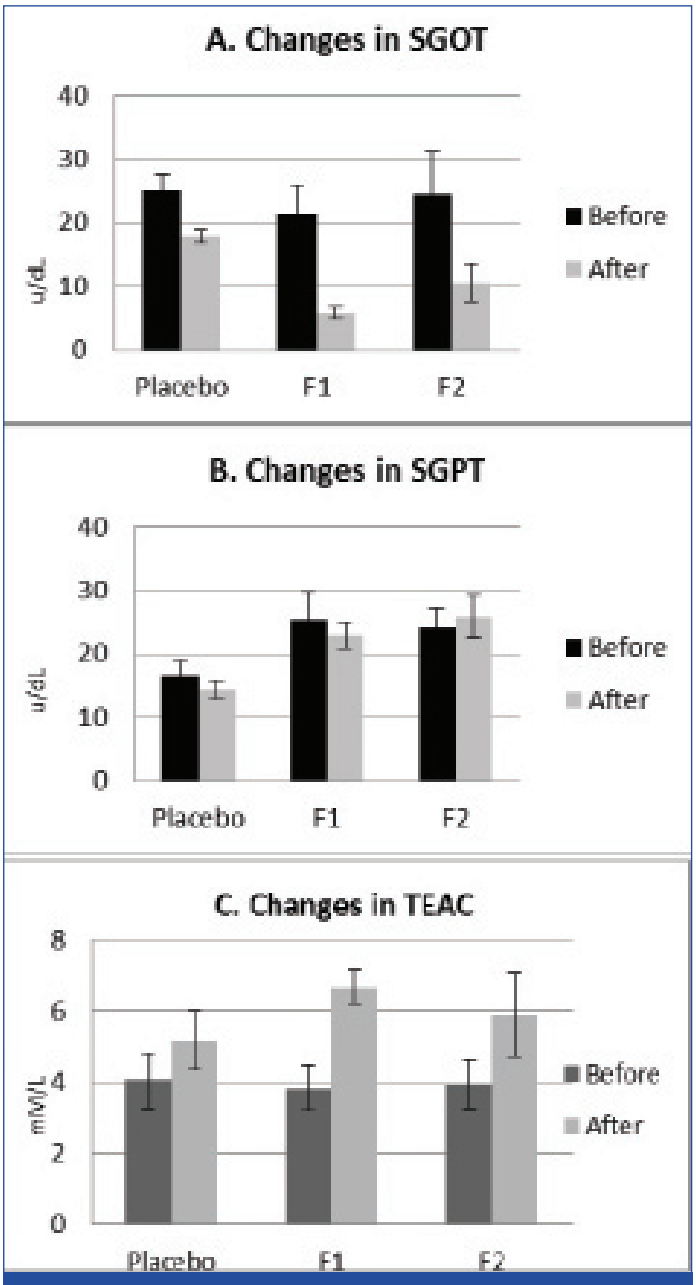
[Table/Fig-8] also shows the changes in antioxidant status related parameters due to the effects of F1 and F2. Effect of supplement F1 was significant for all the four antioxidant parameters. Time by treatment interaction terms between F1 and placebo were significant for MDA, TEAC and SOD. In case of F2, changes over time were significant for all the four antioxidant parameters, differences between placebo and F2 were significant for GSH and interaction terms between F2 and placebo were significant for MDA and TEAC.

DISCUSSION

In this paper, the effect of two experimental health beverages was studied in healthy male and female volunteers, while, control group consumed a beverage with artificial flavour and colour and without any fruit juices. The key features of this study were the improvement in parameters related to lipid profile and antioxidant status by

Source of variation	Biomarker	F	p-value
Supplement	GSH	5.544467	0.028868
Time (days)		58.92437	2.19E-07
Interaction		1.277445	0.271748
Source of Variation		**F**	**p-value**
Supplement	MDA	2.095748	0.158815
Time (days)		66.16101	7.43E-09
Interaction		12.92142	0.001231
Source of Variation		**F**	**p-value**
Supplement	SOD	0.060152	0.808044
Time (days)		5.16153	0.030971
Interaction		1.012959	0.322814
Source of Variation		**F**	**p-value**
Supplement	TEAC	0.438461	0.512083
Time (days)		2.445843	0.126586
Interaction		3.060216	0.088753

[Table/Fig-7]: ANOVA Comparison of placebo vs F2.
GSH:reduced glutathione; MDA: Malonedialdehyde; SOD:Super Oxide Dismutase; TEAC: Trolox Equivalent Antioxidant Capacity



[Table/Fig-8]: Effects of formulations (i.e., FI, F2 and Placebo) on: a) SGOT; b) SGPT; c) TEAC.

the supplements with little or no effect on the glucose response. The second important finding of this study was that of significant increase of Hb percentage by F2 and decrease in SGOT by F1.

Most of the research on functional food development has been from Western countries and the product usually has a single functional quality. However, present antioxidant formulations are multi component and seem to have multifunctional activity. This could also be the result of blending different fruit juices/plant extracts. An addition of herb extracts may have further enhanced the nutraceutical potential of the formulation F1.

Dyslipidemic condition is present when either elevated triglycerides (>150 mg/dL) or elevated cholesterol (>200 mg/dL) or elevated LDL (>130 mg/dL) exist [15]. Indians are known to be predisposed to dyslipidemia [16]. In the present case, being the phase one trial, subjects were healthy and majority had lipid profile parameters within normal range. Yet, a lipid lowering effect was manifested. Effects of supplementing F1 and F2 in dyslipidemic subjects might better manifest their lipid lowering potential.

Iron deficiency anemia is rampant in Indians especially in women of child bearing age irrespective of socio economic class. Supplementing F2 in present study has resulted in small but significant rise of Hb by 1.9%. This could be due to organic acids and ascorbic acid present in F2.

There was also a favourable effect of formulations on levels of antioxidant enzymes like, CAT, SOD and liver function biomarker enzymes (SGOT) along with decreased MDA and increased TEAC and GSH. These results support the antioxidant and liver protection potential of formulations which needs to be further studied. Two-way ANOVA which reveal the contrasts between outcomes of experimental formulations against placebo have shown improvement in antioxidant related parameters by both F1 and F2.

Blending of various juices and extracts has been successful in achieving significantly higher antioxidant and biological effects and also offered better taste and flavour resulting into acceptability for long term consumption [17-19]. But, there are limited human studies on nutraceutical effects of blended beverages. Present study was planned to substantiate some of the in vitro claims for the constituent juices and their mixtures. Synergism between the active ingredients of component fruits used in the study was likely to be present for their net result on the outcomes. This was also noticed during the in vitro experiments done prior to this study.

The hypoglycemic and lipid lowering effect of 2 or 3 gm amla (*E. officinalis)* powder per day indicated a significant decrease (p-value < 0.05) in fasting and two hour postprandial blood glucose levels on the 21st day in both normal and diabetic subjects along with significant (p-value <0.05) decreases in total cholesterol, LDL and triglycerides [19]. In the present data, for F1, fresh juice of raw amla fruits was used as the major ingredient which was considered to be more effective than dry powder. F1 supplementation has also resulted in significant changes in total cholesterol, HDL and LDL levels in young healthy adults confirming the lipid lowering action. The absence of effect on plasma glucose levels in F1 might be due to added sugar. Phenolics present in foods and herbs are of great interest since last two decades as evident from their favourable effect on human health. However, this has been based on epidemiological studies wherein an negative association has been shown with the intake of polyphenol rich foods and the incidence of non communicable diseases [5]. Results of F1 which is rich in polyphenols suggest its promise as antioxidant supplement.

Holy basil or *O. sanctum* has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardio protective, analgesic, antispasmodic and adaptogenic actions [20]. In the present study the juice of fresh leaves has been used to preserve its nutraceutical potential. Secondly, fresh juice of Holy basil also contains a fragrance due to essential oil which improved sensory value of F1.

Bael fruit and bael leaf are not commonly consumed as fruit or as leafy vegetable due to their bitter taste but are known in Ayurveda for their medicinal properties like antidiarrheal action and antidiabetic effects. We have previously reported very high antioxidant potential of bael fruits [19] based on in vitro assays. The fruits are seasonal, grown as wild fruits and dry powder of fruits is used as medicine. jamun fruits are found both in forests and gardens. Antidiabetic activity of the seed kernel of *syzygium cumini linn* is known [19]. But our data showed promising antioxidant potential in fruits. This was the reason for including bael fruit powder extract and jamun juice in F1. One study on Thai health beverages to determine antioxidant capacity, total phenolics and sugar content of 12 pasteurized and sterilized has been reported. Bael fruit drink had the significantly highest total phenolic compounds and there were significant associations of total phenolics with the antioxidant capacity [21]. This indirectly supports our findings.

Black grape variety (*labrusca X vinifera*) was used in F2 as main ingredient. This has been reported by us to be having highest antioxidant potential among 12 varieties and their hybrids [12]. This variety has been released in India. Other ingredients of F2 viz., pomegranate, grape fruit, guava and kokum have also been previously studied by us for their in vitro antioxidant potential [13]. It was of interest to study and confirm their combined effect in a human trial.

LIMITATION

Although, 48 subjects entered the study initially, only 42 subjects completed the study. Further studies on patients with dyslipdemia need to be done with larger sample size. Another limitation of the study was lack of evidence for increase of vitamin C levels due to the consumption of F1.

CONCLUSION

Both the formulations had a favourable effect on lipid profile of young healthy subjects. Amla based beverage showed an improvement in liver function as seen from SGOT levels. Grape based formulation resulted in an increase of Hb levels which is of significance for Indians. Both the beverages showed a promise as antioxidant supplements, indicated by biomarkers like SOD, MDA and TEAC. The formulations seem to be promising as the functional food products. These will have value as health beverages at international level and also for Indians as economical substitutes for imported formulations.

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