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RESEARCH REACH

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**RESEARCH REACH
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THERAPEUTIC VALUES OF SPIRULINA

Subhadra K and Subbulakshmi G

College of Home Science, Nirmala Niketan,
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Spirulina is a microscopic blue green aquatic plant and is the most complete and richest source of whole array of nutrients. It is possibly the single source of all the nutrients needed for a strong immune system and thus prevents many degenerative diseases. If cultivated under well-controlled conditions, at very low concentrations in the diet, spirulina has been shown to possess therapeutic value on a variety of disorders ranging from hypercholesterolemia to cancer.

Search for newer sources of protein started three and half decades ago when shortage of protein in developing countries received global attention. Various unconventional proteins such as Oil Seed Protein, Leaf Protein Concentrate (LPC), Fish Protein Concentrate (FPC) and Single Cell Protein (SCP) were considered for the eradication of protein malnutrition. The term SCP was coined in 1966, which includes protein originated by unicellular or simple multicellular organisms such as Bacteria, Yeast, Algae and Fungi. Protein from algae is also termed as Bio Mass Protein (BMP). The difficulties and cost constraints in the production of LPC from foliage led to the development of suitable technologies for the cultivation of algae. Research on algae received greater emphasis with the launching of the "Algae for Feed and Food" programme under an Indo German Agreement at Central Food Technological Research Institute (CFTRI), Mysore in 1973 and also by the launching of the All India Co-ordinated Project on Algae (ACOPA) under the Department of Science and Technology (DST), India in 1976. Over and above, the research work carried out on Algae at Murugappa Chettiar Research-Centre (MCRC), Madras also has added to the knowledge on the hidden wealth on earth.

Scenedesmus, Chlorella and Spirulina are some of the algae that are considered good sources of nutrients. Among these varieties Spirulina, cultivated in clean water is the most ideal alga for human consumption (Venkataraman 1980). Spirulina is a blue green alga (Cyanobacteria) and contains a blue pigment called phycocyanin. It has spirally twisted filaments (hence the name 'Spirulina') with a length of 300-500 microns and width of 8 microns. The most commercialized spirulina species are *Spirulina platensis*, *S. maxima* and *S. fusiformis*. Consumption of spirulina as human food has a historical back ground. Spirulina has been consumed in the form of sauce called 'Dihe' by the Kanembou Tribe, North of lake Chad in Central Africa (Delpeuch et al 1975). 'Tecuitlatl' was eaten by Aztecs in ancient Mexico. Today spirulina has found its place in the diets of athletes, lactating women and obese persons (Venkataraman 1993).

The cultivated spirulina has a good potential in the health food market as a healthy wholesome food and also as a source of nutraceutical products such as β -carotene, Polyunsaturated Fatty Acids etc. The world's production of spirulina has been reported to be around 3,360 metric tonnes per year according to the 1999 report. The cultivated algae can be either drum dried, spray dried or even sun dried. However, the nutritional value or the chemical composition of the alga was found to be influenced to some extent by the method of drying (Table-1). The Protein Efficiency Ratio (PER), Digestibility Coefficient (DC), Biological Value (BV) and the Net Protein Utilisation (NPU) of the drum dried

Scenedesmus and spirulina algae were found to be significantly higher than the products dried by the other two methods (Becker and Venkataraman 1978).

TABLE 1. PROTEIN QUALITY OF SPIRULINA

Alga Variety	Processing method	PER	DC	BV	NPU
Senedesmus acutus	Sun dried	1.27	72.5	72.1	52.0
	Drum dried	2.24	81.4	80.4	65.8
Spirulina platensis	Sun dried	1.71	83.3	77.6	65.0
Casein (Reference protein)	--	2.70	98.08	87.76	83.54

Venkataraman et al 1977, Venkataraman 1980.

Nutritional / Chemical Composition of Spirulina:

World Health organization (WHO), and scientists in the United States, France, West Germany, Mexico, Vietnam and Japan have reported that spirulina is one of the best sources of nutrients quantitatively as well as qualitatively (Table 2).

Spirulina has a soft cell wall made of complex sugars and protein, and is different from most other algae for its easy digestibility. The chemical composition of spirulina proves that it is a high protein, low fat and low carbohydrate containing nutritious alga. The major calorie source in spirulina is its protein (65%-71%). Spirulina contains more protein as compared to other plant and animal foods. Dried Spirulina contains up to 70% protein by weight (Dillon et al 1995). Poly-beta-hydroxybutyrate has also been identified in the cyanobacterium Spirulina platensis (Campbell et al 1982).). It is also a source of gamma linolenic acid (GLA) along with linoleic and arachidonic acids

TABLE 2. PER CENT NUTRIENT COMPOSITION OF SPIRULINA

Nutrient	Amount	Nutrient	Amount
Moisture (g)	7.0	Carotene (mg)	190.0
Protein (g)	71.0	Vitamin E (mg)	19.0
Ash(g)	9.0	Cyanocobalamin	0.2
Crude Fibre (g)	0.9	Thiamin (mg)	5.5
Chlorophyll (mg)	760.0	Riboflavin (mg)	4.0
Inositol (mg)	35.0	Nicotinic Acid (mg)	11.8
?-linolenic acid (mg)	1.3	Pyridoxine (mg)	0.3
Xanthophylls (mg)	180.0	Biotin (mg)	0.04
Calcium (mg)	132.0	Iron (mg)	58.0
Zinc (mg)	3.9	Phosphorus (mg)	894.0

The quality of spirulina protein is superior to that of other proteins and only slightly

lower than that of casein. Eighteen amino acids including all the essential amino acids are present in spirulina (Table 3). It has an unmatched digestibility coefficient of 95 %. The amino acids in spirulina are delivered in an essentially "free form" for almost instantaneous assimilation. Omsted et al (1973) and Vande Decken et al (1974) showed that protein in spirulina was complete with all essential amino acids and hence could support the growth of animals without any EAA substitution but further improvement in the Biological Value and NPU was observed with methionine enrichment (Narasimha et al 1982). Bourges et al (1971) reported a PER value of 2.2 for Mexican spirulina which is only slightly lower than that of casein (2.78). However, processing of spirulina that involves various physical and chemical treatments do not affect the DC (73-78%) of spirulina (De Hernandez and Shimada 1978). Growth studies with algal protein showed increased body weight gains at 10 and 15% levels of incorporation (Anasuyadevi et al 1979). Improved PER was also reported with spirulina (*S.platensis*) supplemented cereal diets by Anasuyadevi and Venkataraman (1983). Similar results were obtained with *S.fusiformis* supplemented rice diets as compared to casein diets even though the NPU of *S.fusiformis* was reported as only 78% of that of casein by National Institute of Nutrition and hence the beneficial effect of *S.fusiformis* might be attributed to the quantity of the alga consumed by the animals (NIN Report 1988). Several human trials were also conducted in order to explore the nutritional and health benefits of spirulina. A positive Nitrogen balance was achieved in malnourished adults who were fed on spirulina tube feed (Santier 1978). Chamorro from UNIDO in 1980 declared spirulina as a superior source of nutrients as compared to soya in clinical performance.

TABLE 3. ESSENTIAL AMINO ACID CONTENT OF SPIRULINA

Amino acid	FAO Pattern	Egg protein	Spirulina
Valine	5.0	6.0	7.5
Leucine	7.0	8.6	10.4
Isoleucine	4.0	5.4	6.4
Phenylalanine & Tyrosine	6.0	9.3	10.1
Lysine	5.5	7.0	4.4
Methionine & Cystine	3.5	5.7	3.6
Tryptophan	1.0	1.7	0.8
Threonine	4.0	4.7	5.4

*Becker and Venkataraman (1978).

Spirulina is a natural source of essential fatty acids as 10 grams contain an average of 225 mg of essential fatty acids in the form of linolenic acid and GLA (Table 4).

TABLE 4. ESSENTIAL FATTY ACID COMPOSITION OF SPIRULINA*

Fatty Acids	Percent (%)
Myristic acid	0.2
Palmitic acid	45.0
Palmitoleic acid	5.6
Heptadecanoic acid	0.3
Stearic acid	1.4
Oleic acid	2.2
Linolenic acid	17.9
Gamma linolenic acid	24.9
Others	2.5
Total	100

* Dillon et al 1995

Besides macronutrients, spirulina contains high levels of various vitamins, and minerals like calcium, iron, magnesium, manganese, potassium, and zinc (Table 2), high amount of RNA and DNA and chlorophyll (<http://www.nutrimart.com/library.htm>). It is one of the richest vegetarian sources of B-carotene, which is 20 times that of carrot and 26 times richer than raw beef liver. The bioavailability of B-carotene and Riboflavin was also found to be superior as compared to their absorption from elemental forms (Annapurna et al 1991). But Kapoor and Mehta (1991) documented that the bioavailability of these nutrients was similar to that from the pure substances. It is also the only richest vegetarian source of B12, a nutrient otherwise found almost exclusively in animal foods. Vitamin B12 is essential for normal growth and neurological function. Spirulina is the richest whole food source of vitamin E. It is 3 times richer than raw wheat germ, and is highly biologically active. Thus spirulina has an excellent blend of fat soluble (Vitamins A and E) and water-soluble vitamins (B-complex and Vitamin C). Besides being a rich source of calcium, phosphorus, potassium and sodium, spirulina contains high iron content which is 12 times that of any other food (Becker and Venkataraman 1978). Iron in spirulina is 58 times richer than that of raw spinach. Johnson and Shubert (1986) demonstrated that the absorption of iron was higher from spirulina than ferrous sulfate diet. The highly available iron and folic acid in spirulina increased the serum iron and haemoglobin levels in both animals and humans. A significant rise in the serum iron and haemoglobin levels were reported in the rats fed on spirulina based diet (Kapoor and Mehta 1991). Consumption of 4g of spirulina per day after meals increased blood haemoglobin content of hypo chromic anaemic women from 10.9-13.2 grams (Takeuchi et al 1978). Studies conducted on other animals such as poultry (Brune 1982),

silkworm (Mathavan et al 1984), and calf (Amrith kumar et al 1980) showed growth and/or yield promoting characteristics by spirulina and thereby its economical benefits.

Bioavailability of various nutrients in spirulina has been reported to be excellent by several studies conducted on both animals and humans and due to this fact spirulina could be considered to meet the recommended Dietary Allowances of these nutrients. Spirulina has high biological value protein with a superior complete amino acid profile containing all 8 essential amino acids and 10 non-essential amino acids with the correct proportions. It has an unmatched digestibility coefficient of 95%. The amino acids in spirulina are delivered in an essentially "free form" for almost instantaneous assimilation. Omsted et al (1973) and Vande Decken et al (1974) showed that protein in spirulina was complete with all essential amino acids and hence could support the growth of animals without any EAA substitution and further improvement in the Biological Value and NPU was observed with methionine enrichment (Narasimha et al 1982). Bourges et al (1971) reported a PER value of 2.2 for Mexican spirulina which is only slightly lower than that of casein (2.78).

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Vitamin B12 in spirulina provides energy, and is essential for normal growth and neurological function. Iron in spirulina is 58 times richer than that of raw spinach. Johnson and Shubert (1986) demonstrated that the absorption of iron was higher from spirulina than ferrous sulfate diet. The highly available iron and folic acid in spirulina increased the serum iron and haemoglobin levels in both animals and humans. A significant rise in the serum iron and haemoglobin levels were reported in the rats fed on spirulina based diet (Kapoor and Mehta 1991). Consumption of 4g of spirulina per day after meals increased blood haemoglobin content of hypochromic anaemic women from 10.9-13.2 grams (Takeuchi et al 1978).

The bioavailability of β -carotene and Riboflavin was also found to be superior as compared to their absorption from elemental forms (Annapurna et al 1991). But Kapoor and Mehta (1991) documented that the bioavailability of these nutrients was similar to that from the pure substances. Studies conducted on other animals such as poultry (Brune 1982), silkworm (Mathavan et al 1984), and calf (Amrith kumar et al 1980) showed growth and/or yield promoting characteristics by spirulina and thereby its economical benefits. Several human trials were also conducted in order to explore the nutritional and health benefits of spirulina. A positive Nitrogen balance was achieved in malnourished adults who were fed on spirulina tube feed (Santier 1978). Chamorro, UNIDO (1980) declared spirulina as a superior source of nutrients as compared to soya in clinical performance.

Safety Issues:**Toxicological Studies:**

Spirulina by its nature is a mixture of species and strains on which several toxicological studies have been carried out by Chamorro (1980). The UN industrial Development Organisation report based on Mexican spirulina says " Speaking generally it may be concluded that spirulina when administered at 10, 20 & 30% levels in the diet does not produce any variation in the parameters studied during researches on sub-acute toxicity, reproduction and lactation, mutagenesis and teratogenesis". Further no second or third generation reproduction, fertility, lactation or birth defect problems, cancer causing properties, heavy metal, pesticide and bacterial toxicity were found. Chamorro and Salazar (1990) and Salazar et al (1996) investigated the embryotoxic and fetotoxic potential of spirulina with negative results. Venkataraman (1980) also reported the safety of spirulina on the histopathological and biochemical parameters of rats fed on spirulina at 10 and 15% levels as sole protein source. Krishnakumari et al (1981) also reported the absence of oral or dermal effects with spirulina in rats. No abnormalities in the organ weights of kidney, heart, liver and spleen were observed in rats fed on spirulina for over 12 weeks (Becker and Venkataraman 1982). Bourges et al (1971) proved that spirulina rich diets (260 & 730 g / Kg of diet) showed good survival and health with normal microscopic and macroscopic picture of organs. Yoshino et al also proved haematological and histological non-toxicity of spirulina, in 1980.

Spirulina itself appears to be non-toxic. A study on rats showed that high Spirulina intake caused no weight reduction or toxicity symptoms in rats, nor did Spirulina affect the rats' ability to reproduce normally (Dillon et al 1995; Jassby 1988). These researchers suggest that it is not prudent to eat more than 50 g of Spirulina daily. The reason they give is that the plant contains a high concentration of nucleic acids, substances related to DNA. When these are metabolized, they create uric acid, which could cause gout or kidney stones. This is of special concern to those who have already had uric acid stones or attacks of gout. Ingestion of spirulina in small doses over a long period has shown to be tolerable in normal subjects (Santier and Tremolieres 1975). Maximum safe doses of Spirulina in pregnant and nursing women, young children, and individuals with kidney or liver disease have not been determined.

Therapeutic Dosages:

Researchers studying effects of Spirulina on health have used a variety of doses, ranging from 1 to 8.4 g daily and a dosage of 50 g daily is generally regarded as the maximum safe dose (Slotton et al 1989).

Organoleptic acceptability:

Spirulina fusiformis was found to be well acceptable at 5 and 10% levels of incorporation in different recipes (Umesh and Seshagiri 1984) whereas good acceptability was also reported by Kapoor and Mehta (1991) at 15% level of incorporation. Spirulina is being marketed in different forms, recipes and products and a large number of formulations are available for the preparation of foods using spirulina powder including fermented foods comparable to cheese, yoghurt, tofu etc. Decolorized, odorless and tasteless spirulina concentrates are being considered for the preparation of novel foods (Venkataraman 1993).

**FOOD GRADE SPECIFICATIONS OF INDIAN STANDARD ALGA SPIRULINA
(IS 12895: 1990 AMENDMENT NO.1 JUNE 1991) ***

Characteristic	Requirement
Moisture percent by mass, Max	9.0
Total Ash (on dry basis)	9.0
Acid insoluble ash (on dry basis) percent by mass, Max	0.5
Protein percent by mass,	55
β -carotene mg/100g. Min	160
Phosphorus mg/100g. Max	850
Iron mg/100g. Max	100
Zinc mg/100g. Max	35
Potassium mg/100g. Max	1350
Lead mg/kg. Max	2.5
Arsenic mg/kg. Max	1.1
Cadmium mg/kg. Max	1.0
Mercury mg/kg. Max	0.1
Coliform bacterial count/g. Max	Absent (in 1g)
Salmonella sp.	Absent (in 1g)
Shigella	Absent (in 1g)
E.coli	Absent (in 1g)

*Seshadri and Jeeji Bai (1992)

Therapeutic / clinical benefits of Spirulina:

- Stimulates immune system to destroy invading disease organisms and carcinogens.
- Potentiates the immune system with its anti-tumor, antiviral and interferon inducing effects.
- Helps to sanitize the bowel by detoxifying the colon and promoting the growth of friendly bacteria.
- Promotes tissue repair in wounds and burns and has anti-infectious properties.
- Decreases cholesterol levels and helps to lower the risk of cardiovascular disease.
- Works as an anti-inflammatory agent, helping to reduce the inflammation characteristic of arthritis.
- Provides superior nutritional support for anyone who is weakened by disease, alcohol or drug abuse.
- Helps to balance RNA and DNA nucleic acids.
- Curbs the appetite and helps to stimulate the metabolism. (www.spirulina.com)

Hypocholesterolemic Effect:

The cholesterol lowering effect of spirulina has been well studied in animals as well as humans (Chen et al 1981; Kato et al 1984; and Anasuya Devi and Venkataraman 1983). Administration of 4.2 grams of spirulina per day for a period of 8 weeks showed greater reduction in serum total cholesterol level in adult human subjects especially with higher initial levels and resulting in improvement in their atherosclerotic index (Nahaya et al 1986 and 1988). Iwata et al (1990) also reported the increase in lipoprotein lipase and thereby lipid-lowering effect of spirulina in fructose induced hyperlipidaemic rats. Thirty healthy male mild hyperlipidaemic and mild hypertensive adult volunteers were supplied with 4.2g of spirulina per day for eight weeks. Significant reduction in the total serum cholesterol was found during the treatment but the levels returned to base line after the supplementation was discontinued. The effect was more marked in hypercholesterolemic and high cholesterol consuming subjects. Only a slight increase in the HDL-C was observed with no change in the serum triglycerides and body weight. There were no adverse effects either (Nakaya et al 1988).

Evidence from animal studies and one small controlled (but not blinded) study in humans suggests that Spirulina might help in lowering cholesterol (Iwata et al 1990; González de Rivera et al 1993). However, in the absence of double-blind placebo-controlled trials, it is too early to say that Spirulina can be used for this purpose. Spirulina is the richest whole food of Gamma Linolenic Acid (GLA) that helps lower blood cholesterol and high blood pressure and eases such conditions as arthritis, premenstrual pain, eczema.

Hypoglycemic effect:

Studies on the hypoglycemic effect of spirulina are very limited. In a study conducted by Dinesh Babu (1989) five male and five female Non Insulin Dependent Diabetic patients showed a significant reduction in the blood sugar levels upon a six-week supplementation with Spirulina (1.8 g / day).

Weight Loss:

Manufacturers of Spirulina supplements sometimes claim that the plant can reduce appetite, thereby helping overweight individuals control their food intake. In a double-blind cross-over study Vs placebo supplementation of 2.8 grams of Spirulina thrice a day over a period of 4 weeks showed a small but statistically significant weight loss in obese out patients (Becker et al 1986). Larger and longer studies are needed to establish whether Spirulina is indeed an effective treatment for obesity.

Anti-Cancer Agent:

Some common forms of cancer are thought to be a result of damaged cell DNA running amok, causing uncontrolled cell growth. Cellular biologists have defined a system of special enzymes called Endonuclease which repair damaged DNA to keep cells alive and healthy. When radiation or toxins deactivate these enzymes, errors in DNA go un-repaired and, cancer may develop. In vitro studies suggest the unique polysaccharides of Spirulina enhance cell nucleus enzyme activity and DNA repair synthesis (www.healthrevolution.com). Preliminary evidence suggests that Spirulina, like other nutritious plant foods rich in protein, B-carotene and other micronutrients may help prevent cancer (Underwood 1984; Mishima et al. 1998). Spirulina phycocyanin treatment increased the rate of survival of rats with liver

cancer Topical application of an extract of *Spirulina - Dunaliella*, three times weekly for 28 weeks inhibited the tumour development and facilitated regression of buccal pouch cancer in hamster and humans (Schwartz et al 1988). Supplementation of *Spirulina fusiformis* (1g/day for 12 months) which is well known for its high content of vitamin A to pan tobacco chewers suffering from oral leukoplakia in Kerala resulted in a statistically significant complete regression of the lesions in 45% evaluable subjects (n=44) as compared to 7% regression in the placebo group (n=43). However, recurrence of the lesions was observed after one year in 9 out of 20 complete responders on discontinuation of the supplement (Mathew et al 1995). A better control of the cell functions and thereby prevention of generation of malignancy with spirulina phycocyanin treatment was reported by DIC, in 1983. A significant reduction in the hepatic cytochrome P-450 content and induction of hepatic Glutathione S- Transferase without any change in the extra hepatic enzyme activity was observed in the spirulina supplemented Swiss Albino Mice which proved the modulatory potential of *Spirulina fusiformis* (Mittal et al 1999). Thus several studies have proved the inhibitory effect of spirulina on carcinogenesis.

Pro-biotic:

The probiotic effect of spirulina has been proved by Tokai et al (1987) in a study on rats. A 327% increase in the *Lactobacillus* content, 45% increase in vitamin B1 level in the caecum of the rats indicated the positive effect on the absorption of thiamine from the diet. The presence of probiotic compounds and their positive effects on health has also been confirmed by Kay (1991) and Dillon (1995).

Anti-HIV:

Test tube and animal studies suggested that *Spirulina* might have some activity against the HIV virus, but much more research needs to be done before we could say that *Spirulina* is helpful against HIV infection (Aychunie et al., 1998; Hayashi et al 1996). "Water extract of *Spirulina platensis* inhibits HIV-1 replication in human derived T-cell lines and in human peripheral blood mononuclear cells. A concentration of 5-10 µg/ml was found to reduce viral production!" (www.healthrevolution.com).

Immunological functions:

Spirulina has been proved to boost the humoral and cellular immune systems by accelerating the production of antibodies and cytokines (humoral immunity) which protect the body from harmful microbes. *Spirulina* also increases the synthesis and potential of T-cells, Macrophages, B-cells and the anti-cancer Natural Killer cells (cellular immunity) and protects the body from the effects of environmental and biological toxins as well as infective agents (www.healthrevolution.com).

Dietary *Spirulina* (*S.platensis*) has been shown to bring about enhancement of chicken macrophage phagocytic function and nitrite production (Al-Batshan et al 2001). Possible evidence of the protective effects of *Spirulina* towards food allergy and microbial infection was found by Hayashi et al (1998).

Preliminary evidence suggests that *Spirulina* or other blue-green algae products may activate the immune system, counter allergic reactions, help protect the liver from toxic chemicals, reduce blood pressure, and help control symptoms of ulcerative colitis (Hayashi et al 1994, Yang et al 1997).

Galactogogic agent:

A significant increase in the litter weight and milk content were observed and reported by Venkataraman (1991) on the supplementation of spirulina at 500 ppm level to pregnant CFT-Wistar rats from 14th day of gestation to 14th day of post natal period thus proving the galactogogue effect of spirulina in animals. A practice of selling spirulina as as lactogil to increase the milk production in nursing mothers has been observed in Vietnam. Spirulina supplementation during pregnancy (from the 24th week of gestation until delivery) increased the serum retinol levels of the subjects significantly and also resulted in slightly higher birth weights of the neonates as compared to the body weights of the neonates born to control subjects (Mridula and Subbulakshmi, 2000).

Anti- Anaemic agent:

Spirulina is the only plant source of highly available iron content. Takeuchi et al (1978) have demonstrated the haemoglobin rising capacity of spirulina (21%) by feeding 4 grams of spirulina after each meal to eight anaemic women for a month. In another human trial, an average increase in the haemoglobin level by 1.33 gm/dl with the supplementation of 2 grams of spirulina a day for 36 days has been reported by Seshadri and Valliammal (1980). A highly significant increase in hemoglobin levels ($p=0.002$) was seen in track and field athletes on Spirulina supplementation, as compared to the placebo group. The mean increase in hemoglobin was greater in females who had very poor Haemoglobin status initially than in males. Possibly, the iron supplementation was of greater benefit to them due to their low hemoglobin levels at baseline. The increase in hemoglobin could be due to the high iron content of Spirulina (58 mg%). Puyfoulhoux et al (2001) observed a high bioavailability of iron from Spirulina (6.5 times that of iron from meat) and concluded that Spirulina could represent an adequate source of iron. In a study by Kapoor and Mehta (1998), Spirulina appeared to be effective in improving the iron status of rats during pregnancy and lactation. Diets containing Spirulina alone or in combination with wheat gluten resulted in significantly higher iron storage and hemoglobin contents in these rats.

In addition to the above mentioned therapeutic uses, Spirulina was also found to possess various other clinical benefits such as (a) prevention of oxidative stress due to its antioxidant capacity (Shenoy et al 1991), (b) Hepatoprotective effect due to its drug detoxification capacity (Juvekar and Nimbkar (2000), (c) Prevention of nephrotoxicity caused by heavy metal poisoning (Yamane, 1988), (d) A radio protective effect (Spir.Abstr.) and (e) prevention of incidence of infections such as conjunctivitis by improving the immunity (Seshadri 1993). Belay and Ota (1993) have summarized the therapeutic effects of spirulina and suggest further clinical research on the radioprotective effect and hypolipidaemic and hypoglycaemic effect of spirulina.

Besides being a nutritious product, spirulina has found its place in the cosmetic industry as a skin care product due to its valuable vitamins (Venkataraman 1989) and the phycocyanin from spirulina has been used as a natural colouring agent.

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EFFECT OF EUCALYPTUS OIL AS FUMIGANT AGAINST PULSE BEETLES ON GREEN GRAM SEEDS

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The present study was planned with the objective of saving green gram seeds from pulse beetle (*Callosobruchus chinensis*) with eucalyptus oil, which could be used as fumigant owing to its volatile nature and insecticidal potential. Freshly harvested and disinfested pulse seeds were fumigated with three doses of eucalyptus oil (0.000, 0.010, 0.015, 0.020ml / bin) and stored for 6, 12 and 24 weeks separately in airtight aluminum bins. Development of the test insect was completely arrested except few eggs on the seeds at the highest dose used (0.020ml / bin). Therefore green gram seeds fumigated with this effective dose were further analyzed for nutritional quality and consumer acceptability before and after each storage period. It was observed that oil vapors were able to reduce the pace of biochemical changes which otherwise was higher in control sample. But storage period had an impact. Consumers rated the cooked fumigated samples as good.

Green gram is one of the most widely consumed pulses for different purposes. This legume is harvested both in summer and winter seasons and highly prone to get infested with bruchids or pulse beetle causing extensive damage to the quantity and quality of the seeds. It has been estimated that about 8.5 % get lost during post harvest handling and storage in India (Anon, 1990). The infestation is usually carried to stores from the field itself and multiplies fast under conducive environment; therefore it is advisable to treat the seed just after harvest and storage to prevent development of the beetle.

Several chemical or plant based protectants are being used to protect pulses from ravages of bruchids throughout the world. Most of them are plant powders, plant extract, vegetable oils, dust, ashes, or sprays. They have been found to pose some limitations in application or in effectiveness. Fumigation is one of the most effective and worldwide acceptable methods to protect grains from insect pests during storage. Effectiveness of the method lies in the fact that the vapors of the fumigant soon get dissipated into the enclosed area of store and attacks the target insects in the container and destroy them. Synthetic chemicals are generally being used as fumigants. Though effective against stored grain pests, they leave residues, which may have adverse effect on human health.

There are quite a few natural sources such as essential oils drawn from aromatic or medicinal plants, which can serve as fumigants. They are volatile in nature and possess tremendous insecticidal potential (Agarwal et al 1988). Essential oils like mint, eucalyptus, citrus and *Acorus calamus*, etc. have shown vapor toxicity against pulse beetle under laboratory conditions (Mishra et al 1981; Ahmad and Eapen 1986). Eucalyptus oil has been traditionally used as disinfectants, digestives, analgesics, in cold and cough remedies and other preparations of therapeutic uses (Brud and Gora 1989). It can also be safely consumed internally up to a dosage of 2ml per day by adults in dilute form according to Newall et al (1996). It finds its use as an inhaler also.

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Eucalyptus oil vapour has been proved effective by the preliminary experiments against pulse beetle (*Callosobruchus chinensis* Linn.) under laboratory and ambient conditions. Thus the present study was planned to study the impact of this oil vapour as a fumigant on the nutritional quality and consumer acceptability of the stored green gram.

Materials and Methods:

Freshly harvested crop of green gram seeds (var. P-105) was procured from Regional Research Station, Karnal in winters. To ensure that the seeds were free from any hidden infestation, they were given heat treatment at $30^{\circ}\pm C$ temperature for 4 hours as suggested by Mukherjee et al (1968). Different doses of eucalyptus oil i.e. 0.000, 0.010, 0.015, 0.020ml / bin were used. Airtight aluminum bins (1.5 L cap.) were used due to their popularity for storage of grains at household level. Respective dose of eucalyptus oil was dispensed with the help of graduated micro capillary tube on the piece of filter paper enrolled inside the hollow glass tube (2.5 cm long).

- In each bin 400g of clean and infestation free green gram seeds were filled.
- Each bin was labelled for dose, replicate and storage period.
- Insect pairs (10 males + 10 females) were carefully counted and kept in vials.

Insect pairs from vials were carefully transferred into the bin and the glass tube with the filter paper was immersed into the bin containing green gram seeds. Immediately the bin was closed light and sealed with a good quality thick and broad (2.5 cm) cello tape on the closing rim outside stored in triplicates for 6, 12 & 24 weeks separately under ambient conditions (winter months of New Delhi). All the 36 bins prepared similarly were kept on the shelf undisturbed for respective period of storage.

Effect on insect infestation: After respective storage period, each bin was opened at a time and content was sieved so that all the insects (dead and live) fall down. Total number of insects were counted manually and recorded. However, to count the number of eggs in total content, 50 g sample was drawn from each bin and total number of eggs was counted with the help of lens and to get the total number of eggs in each bin this number was multiplied by 8. All the parameters were assessed before and after the storage period.

Moisture content was determined by the International Seed Testing Association (ISTA 1985), the protein content by micro kjeldahl method (NIN 1983), and free fatty acids, reducing and non-reducing sugars by AOAC (1984) methods. Consumer acceptability was assessed by sensory evaluation on the cooked samples according to the method suggested by Amerine et al (1965). A scorecard was prepared for judging different sensory attributes. A panel of 10 judges from Super bazaar Analytical Laboratory and Department of Foods and Nutrition, Lady Irwin College, New Delhi were asked to assign a score (5 = very good, 4 = good; 3 = fair; 2 = poor; 1 = very poor) for each sample for 7 sensory attributes i.e. appearance, consistency, texture, taste, flavor, doneness, and overall acceptability. Care was taken to serve not more than 5 samples at a time.

Results and discussion:

Fumigation with eucalyptus oil has shown to be beneficial on the development and egg laying capacity of the insect, *C. chinensis*. The total number of eggs laid on green gram

seeds decreased with increasing dose of the oil but increased with the period of storage (Table 1). Insect development was completely arrested at 0.015 and 0.020ml / bin leaving 56 and 19 eggs respectively at the end of 24 weeks while the control had 543 insects and 4928 eggs in 400 g.seeds after the same period of storage. Studies have revealed that metabolic activity of the insects slowed down at lower temperature (Howe and Currie 1964) and larvae can also undergo hibernation in winters and resume their activity of developing into adult in warmer months (Rajak and Pandey 1965) and development of *C. chinensis* get prolonged in airtight containers (Singh 1981).

TABLE 1. DEVELOPMENT OF INSECTS AND ITS EGG LAYING CAPACITY DURING STORAGE

Dose(ml/L)	Insect population after				No. of eggs laid after		
	0 wk	6 wks	12 wks	24 wks	6 wks	12 wks	24 wks
0.010	20	0.3	27	80	69	323	1147
0.015	20	0.7	5	0	21	149	56
0.020	20	0.0	0	0	9	80	19
Control	20	2.3	20	543	241	447	4928

* 10 pairs of insect (*C. chinensis*) inoculated at the time laying down the storage experiment were deducted from total numbers observed.

There was close association between moisture content, ambient temperature, relative humidity and the degree of infestation (Pixton 1967; Yadav and Pant 1979). In the present study reduction in moisture content (from 10.5 to 8.3 %) of green gram seeds was observed with the progress in storage period (Table 2). It can be attributed to the effect of oil vapors in airtight containers and low ambient temperature. Control sample showed little higher value (9.2 %) indicating the presence of infestation and an increase in metabolic water of the insects according to Yadav and Pant (1978).

Protein content did not show any marked difference in treated samples before (21.7g) and after the storage (21.3g). However, untreated sample had 22.4g protein at the end of the experiment indicating again that infestation tend to increase the protein content due to the metabolic waste of insects that is nitrogenous in nature. Srivastava et al (1989) have observed similar impact on protein working with mint oil vapours on pigeon pea.

Free fatty acid (FFA) content is considered as an index for grain deterioration (Baker and Zeleny 1957). FFA value increased from 13.7 to 20.2 mg/100 KOH in the experimental samples while the same was 29.7 mg/100 KOH in the control sample because of insect infestation. Similar trend observed by Doharey et al (1983) and Sharma et al (1986) suggested

that this is due to the increased activity of lipase in the grain. It is seen that eucalyptus oil vapours tend to reduce the pace of increase in FFA.

TABLE 2. NUTRITIONAL QUALITY OF GREEN GRAM TREATED WITH (0.02 ml/bin) EUCALYPTUS OIL VAPORS - ON STORAGE.

Parameter		0 week	6 weeks	12 weeks	24 weeks	Mean
Moisture %(g/100g)	Treated	10.5	9.6	9.1	8.3	8.9
	Control		10.1	8.9	9.2	9.4
Protein (%)	Treated	21.7	21.6	21.4	21.3	21.5
	Control		21.3	21.9	22.4	21.9
FFA(mgKOH/100g)	Treated	13.7	14.2	17.7	20.2	17.4
	Control		16.1	29.7	29.7	23.4
Reducing sugar (%)	Treated	1.0	1.1	1.2	1.2	1.1
	Control		1.3	1.2	1.2	1.1
Non-reducing sugar (%)	Treated	4.5	2.1	1.9	1.9	2.0
	Control		2.0	1.6	1.6	1.7

There was slight increase in reducing sugars but sharp decrease in non-reducing sugars (4.5 -1.9%) in 24 weeks of storage. Control had sharper differences. Increase in reducing sugars and an decrease in non-reducing sugars have been observed by Vimala and Pushpamma (1983) and Gupta et al (1984). These changes could be attributed to slower rate of respiration or enzymatic activity in seeds under airtight containers and prevailing ambient temperature and relative humidity.

With regard to the organoleptic acceptability the stored and fumigated samples received more than 3.5 scores for all attributes indicating that dal was rated between good and fair even after 24 weeks (Table 3). Aeration and cooking (preferably in pressure cooker) procedures before consumption could have reduced or removed the effect of eucalyptus oil vapours. However, control sample showed poor performance in all parameters particularly in taste, flavor and over all acceptability and it was obviously due to insect infestation. Highly infested sample was not cooked and served, as it was unsafe for consumption.

Conclusion:

It has been found that 0.02 ml of eucalyptus oil used as a fumigant can effectively control the development of the pulse beetle in green gram seeds on storage without any adverse effect on nutritional quality and consumer acceptability. Further studies on the residual effects on human health is recommended.

**TABLE 3. CONSUMER ACCEPTABILITY SCORES OF
COOKED GREEN GRAM BEFORE & AFTER STORAGE**

Parameter		0 week	6 wks	12 wks	24 wks
Appearance	Treated	3.8	3.5	3.9	4.0
	Control		3.7	3.7	2.7
Consistency	Treated	3.6	3.5	3.7	3.8
	Control		3.5	3.6	2.4
Texture	Treated	3.8	3.4	3.6	3.8
	Control		3.6	3.5	2.1
Taste	Treated	3.7	3.5	3.6	3.7
	Control		3.5	3.4	1.9
Flavour	Treated	3.9	3.3	3.5	3.6
	Control		3.3	3.4	1.9
Doneness	Treated	3.5	3.6	3.6	3.9
	Control		3.7	3.5	2.7
Overall	Treated	3.5	3.4	3.5	3.7
	Control		3.4	3.4	1.8

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GONADAL HORMONES IN SELECTED FOODS

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The present study aims at collecting information on the use of emmenagogic/uterotonic / abortifacient foods across the Hindu communities using rapid assessment survey and identifying the presence of gonadotrophic hormones in selected food or food combinations (F/FC) by Thin Layer Chromatography (TLC). Based on the results of the survey involving 90 elderly women from four Hindu communities, a total of 8 foods including both individual (Dry dates, dry ginger, gingelly seeds & garden cress seeds) & combination foods (Fenugreek seed laddoo, Garden cress laddoo, Omum, jaggery, and shepu seeds mix, Fenugreek seeds, mustard, garlic, dry coconut, black jaggery and ghee mix (FMGCJG mix) were selected for the hormone identification. The petroleum ether & methanol extracts of the selected F/FC obtained by Soxhlet extraction and were subjected to TLC, to identify the presence of testosterone, estrone, 17 α dihydroequilin and medrogestone. Analytical results demonstrated the presence of estrone and 17 α dihydroequilin in dry ginger and medrogestone in the FMGCJG mix. Thus traditional use of these foods for their hormonal effect has been found to be justified.

Gonadotrophic hormones and their analogues have extensive therapeutic applications in reproductive physiology including contraception, menstrual disorders, hormone replacement therapy and abortion. Interestingly, age old traditional practices of consuming certain foods or food combinations in India in these conditions reported to have hormonal influences on female reproductive system. Farnsworth et al (1995) reviewed the presence of β sitosterol in beetroot, estrone in rice, wheat, apple, pomegranate and estrone, estriol and 17 α dihydroequilin in french beans. The past studies on the antifertility effect of foods have revealed resorptive activity in the chicory plant (Prakash and Mathur 1976), anti-implantation in mint leaves (Bodhankar et al 1974) and abortifacient activity in carrot seeds (Saxena 1973).

In the light of these perspectives, there lurks a possibility that foods with steroids, hormones or similar active constituents may influence fertility. According to the folkloric reputation in Nigeria women consumed potash mixed with lime juice, thrice a day for 3 days to terminate a month old conception, but for pregnancies older than a month cactus juice with fresh egg was used. In India women are known to have jaggery with omum (*Trachyspermum ammi*) or ajwain along with generous doses of ghee with the belief that the heat giving effect of the mixture would cause stomach cramps and disturb conception. (Mankekar 1973).

An air of mystery still revolves around such foods & their claims- especially in terms of the active antifertility principle, which remains a relatively uncharted research topic in medical demography & nutrition science. Keeping in mind the innate superiority and mystical aura surrounding the use of such foods, the present study was an attempt to find information, scientific reasoning behind the use of some claimed traditional foods with emmenagogic (agents that induce menstruation) / uterotonic (substances that stimulate the uterus) / abortifacient (capable of expelling the fetus out before term) properties. It also aimed at identifying the presence of gonadal hormones in the selected foods and food combinations.

Materials and Methods :

A rapid assessment survey was carried out on 90 elderly women above the age of 50 years who have been following traditional practices. The sample consisted of 20 Gujaratis, 20 Sindhis, 20 Maharashtrians and 30 South Indians from the Hindu community. From the exhaustive list of foods obtained from above survey, frequently listed foods with emmenagogic / uterotonic / abortifacient claims by majority of the subjects, four individual foods - dry dates (*Phoenix Dactylifera*), dry ginger (*Zinziber officinale*), gingelly seeds (*Sesamum indicum*), garden cress seeds (*Lepidium Sativum*) and four combination recipes with fenugreek seed (*Trigonella oenum graecum*) laddoo, garden cress seed laddoo, and omum + jaggery + dill seeds mixture and a fenugreek seeds + garlic (*Allium Sativum*), + mustard + dry coconut (*Cocos Nucifera*) + black jaggery + ghee mixture) were selected for chemical analyses.

Soxhlet extraction method was employed in a sequential manner to obtain petroleum ether and methanol extracts of above-mentioned foods in duplicates (Khanna et al 1969). Thin Layer Chromatography as described by Sethi (1996) was used. The standards used were testosterone, estrone, 17 α dihydroequilin and medrogestone (a synthetic analogue of progesterone). The sample application device (Linomat IV) was used and visualization of the plates was done in short UV wavelength (254nm). For documentation purpose, the plates were scanned on the basis of densitometric evaluation using CATS software. After scanning, spectra -matching was done to identify the presence of hormones in the food samples.

Results and Discussion :

Ripe papaya was the most commonly listed (71%) food for the emmenagogic/ uterotonic/ abortifacient properties across the various communities. It has been demonstrated in literature that the unripe fruit-pulp has anti implantation activity (Saxena 1973). Besides papaya, several other foods were also reported to have the above-mentioned properties (Table 1).

TABLE 1. FREQUENCY OF FOODS REPORTED AS EMMENAGOGIC / UTEROTONIC / ABORTIFACIENT

Frequency	Foods reported
1-5	Pomegranate, Asafoetida, Cumin, Betel leaves, Omum + jaggery + dill seeds, Mustard + jaggery + coconut + fenugreek seeds + garlic + ghee
6-10	Jaggery, Fenugreek seeds, Mango, Omum, Dry ginger, Jackfruit, Gingelly seeds, Egg, Pineapple, Cashewnut. Omum + jaggery, Fenugreek seed / Garden cress seed laddoo
11-15	Garden cress seeds, Dill seeds, Dry dates, Chicken
16-20	Raw papaya

Pomegranate peel showed antifertility and uterotonicity activities (Dhawan and Saxena 1958). Unripe pineapple was found to show 40% resorptive activity (Prakash et al 1978) and 60% anti-implantation activity in rats (Garg et al 1970). The active principles responsible for the antifertility activity of various foods have been investigated and documented by various scientists (Table-2).

**TABLE-2: ACTIVE PRINCIPLES RESPONSIBLE FOR
ANTIFERTILITY ACTIVITY IN FOODS**

Food	Active Principle	Reference
Papaya	5 hydroxy tryptamine	Paton (1968); Barlow and Khan (1959)
Pineapple	5 hydroxy tryptamine	Paton (1968); Barlow and Khan (1959)
Cuminseeds	coumingine	Abdo and Al-Kafaw (1969)
Betelnut	arecoline	Jacobson (1925); Manske (1955)
	tannin	Saha and Kashinathan (1961); Kumari et al (1964)
Peas	m -Xylohydroquinone	Sanyal (1960)

The alcoholic and aqueous extract of cumin seeds in the concentration of 100-200mg / kg body weight has shown 80-100% anti implantation activity (Garg 1976). The resin of asafetida along with other plant extracts is reported to prevent conception (Das 1966). Betel nut has shown both anti implantation and abortifacient activity (Saxena 1973; Garg et al 1970).

The survey revealed that papaya was unanimously thought to be emmenagogic and abortifacient across the communities. Among the Sindhis there existed a popular belief that egg and a mixture of edible gum + ghee was emmenagogic, dil seeds, garden cress seeds, omum + sugar + ghee were uterotonic, where as omum and dry dates were abortifacients. Some Gujarati participants claimed that dill seeds, fenugreek seeds, omum and garden cress seed laddoo were emmenagogic. They also claimed that garden cress seed laddoo was a uterotonic agent and pineapple, surprisingly enough even tender coconut was an abortifacient. Some Maharashtrais believed that an intake of so-called heaty foods like jackfruit, crab, chicken, cashewnut would cause abortion. They also believed that mango, horse gram broth had emmenagogic and garden cress seeds, fenugreek seeds laddoo, and edible gum had uterotonic activity. The South Indian participants suggested that gingelly seeds were emmenagogic, omum was uterotonic, where as pineapple, chicken, egg, gingelly seeds, colocasia vegetable were abortifacient agents. Some claimed that gingelly seeds and fenugreek seeds (with or without jaggery) were emmenagogic where as omum and fenugreek seed laddoo were uterotonic. Asafetida and amaranth seeds were also reported to be oxytotic by 2 South Indian participants.

It is interesting to note that *Psium sativa*, (peas) with 'm Xylohydroquinone' isolated from the pea oil was experimentally found to reduce pregnancy rates in Indian women (Sanyal 1960). It has also shown similar effects in the Tibetan population, where the staple diet consists of corn and peas (Sanyal 1956). However peas did not figure even once in the foods, listed by participants across the communities and it happens to be one of the commonly used food ingredients among Indians.

In the second part of the study, 4 individual foods and 4 combination recipes (as mentioned in the methodology) were selected for the identification of gonadotrophic hormones. It should be noted that despite a comparatively lower frequency, some combination recipes, were selected for analysis because shepu seeds (*Peucedanum graveolens*), omum, fenugreek seeds, garlic, mustard (*Peucedanum graveolens*), jaggery as individual foods or in various combinations were

repeatedly expressed as having emmenagogic, uterotonic or abortifacient properties by the many participants. The Rf values and I max of both petroleum ether and methanol extracts of gingelly seeds, garden cress seeds, dry dates and omum + shepu seeds + jaggery did not match the Rf values and I max of any of the standard hormones (Table 3). Spectra matching also yielded the same results.

The Rf value and the I max of methanolic extracts of dry ginger matched those of the standard estrone and similarly the values of methanol extracts as well as petroleum ether extract of dry ginger matched with the Rf values and the corresponding I max of 17 α dihydroequilin. This was further confirmed by spectra matching (Table-3).

Among the combination foods, the Rf value and the I max of methanol extract of fenugreek seeds + garlic + mustard + dry coconut + black jaggery + ghee mixture matched those of medrogestone.

Despite the slight deviation in the Rf value and the I max of one of the above mentioned spots [0.33 (320)] from that of the standard, the spectra matching confirmed the presence of the hormone like substances and the deviation was considered negligible. Since this result was not found in fenugreek seed laddoo extracts, containing fenugreek seeds + ghee + sugar, it is logical to consider that ingredients, other than fenugreek seeds and ghee like garlic, mustard, dry coconut or black jaggery may have contributed to the presence of the hormone in the TLC results.

However in the present study, the Rf value and the I max [0.36 (196)] of only one methanol extract of garden cress seed laddoo matched with that [0.35 (196)] of 17 α dihydroequilin. The absence of hormone in the duplicate (extract no. 2) can be attributed to inadequate control in experimental conditions, which may have caused the hormone to drop far below the detection limit of the thin layer chromatography equipment. However referring to the observation that garden cress seeds alone, lacked 17 α dihydroequilin, attributing the presence of the hormone to ghee and yellow jaggery is questionable, therefore reinvestigation of the same is suggested.

Besides the presence or absence of hormones, numerous other constituents of these foods and food combinations may also contribute to the emmenagogic / uterotonic / abortifacient claims but a lacunae in the field of constituent analysis continues to lend an air of mystery to most of these foods.

Conclusions:

In conclusion, the present study reports and confirms the use of foods and food combinations in Indian households with a folkloric belief of their effect on the female reproductive system. Dry ginger was found to have estrone and 17 α dihydroequilin like substances where as the fenugreek seeds+ garlic+ mustard + dry coconut+ ghee + black jaggery mixture demonstrated the presence of a substance resembling medrogestone. However, garden cress seed laddoo needs reinvestigation. Identification of the isolated compounds needs to be done in order to confirm their chemical nature.

TABLE 3. RF VALUES AND I MAX.OF INDIVIDUAL AND COMBINATION FOOD EXTRACTS (E) AND THE STANDARD HORMONES.

	Pet ether extract	Methanolic extract
Foods	Rf (I max)	Rf (I max)
Gingelly seeds	0.62(199)	0.21(286), 0.48(200)
		.27(323), 0.36(322), 0.45(190)
Dry dates	0.80(225)	0.18(287)
Dry ginger	0.26 (287), 0.32 (195), 0.38(198), 0.46(195)	0.26 (198), 0.32 (198) 0.38(198), 0.45 (199) 0.49(198)
	0.26(198), 0.33(198), 0.38(199), 0.45(197)	0.26(198) 0.33(305), 0.38(198), 0.49(198)
Onum+ shepu seeds + jaggery	0.61(198)	0.18(287)
Mustard + jaggery +dry coconut + fenugreek seed + garlic + ghee	0.73(225), 0.75(226) 0.75(225), 0.77(195)	0.33 (320) 0.45(301)
Fenugreek seed ladoo	0.61 (227), 0.62(227)	0.23(281)
Garden cress ladoo	0.41 (201), 0.43(202),	0.36(196)
	0.43 (361), 0.42(361)	0.14(287) 0.14(287)
Garden cress ladoo	0.41(201), 0.43(202), 0.42(361)	0.36(196) 0.14(287)
Standard Hormones		
Testosterone	0.22(251)	
Estrone	0.49(199)	
17adihydroequilin	0.35(196)	
Medrogestone	0.45(300)	

*It should be noted that duplicate values only of foods or food combinations, with the presence of hormone like substances, have been shown in the table.

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SKELETAL DEVELOPMENT OF SCHOOL CHILDREN FROM FLUOROSIS AREAS

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The present study shows the bone development pattern of children aging between 6 to 14 years residing in few villages of fluorosis area of Lathi and Lilia Talukas of Amreli district. It has also been compared with non fluorosis area of Rural Baroda. The fluoride content of drinking water was found to be 2 to 8 ppm in flourish areas. The present study aims at the effect of high fluoride intake on skeletal development of children of fluorosis areas. X-rays of posterior view of both hands including wrists and also anterior-lateral views of elbow of right hand were taken. Radio graphic assessment of ossification centers, size, shape and fusion of carpals, metacarpals and phalanges were noted and thereby the bone age was determined. Length and cortical thickness of second metacarpal bone was also measured. It has been observed that skeletal status of fluorosis area children was better than children of Rural Baroda.

Fluoride is one of the most interesting, biologically remarkable trace elements. It seems desirable to have some fluoride in drinking water and diet to maintain a satisfactory skeletal structure. It was also found that the incidence of dental caries was less in areas where the drinking water contained moderate amounts of fluoride and more in areas where the water contained little i.e. less than 1 ppm or no fluoride. In Gujarat, few villages of Lathi and Lilia Talukas of Amreli district are found to be major fluorosis areas where, approximately, 5-25 % population is affected by fluorosis. Fluoride content of water in Saurashtra region is found to be very high since past few years (2.0 to 8.0 ppm). The present study is an attempt in this direction to find out the effect of high fluoride intake on skeletal development of children between the ages of 6 to 14 years.

Materials and methods:

Subjects chosen for this investigation were mainly healthy children attending primary schools of villages mentioned above, who were having one or more members of family suffering from fluorosis. Children were apparently in normal health except with some signs of dental fluorosis. All the subjects from fluorosis areas under study were taken to General Hospital in Lathi, for X-ray examination. Chronological age of the subjects was noted from school records.

Radiographic technique:

X-ray films obtained from Hindustan Photo Films Mfg. Co.Ltd. were used throughout the study to maintain standard quality. Radiographs of hand and elbow were taken. The x-ray exposure factors for the radiographs were 44-kilo voltage and 8 milliamperere. Posterior view of both the hands including wrist and the anterior-lateral views of elbow of right hand were taken.

Radiographic Assessment:

Radiological appearance of ossification centers, size, shape and fusion of carpus, metacarpals and phalanges were noted and from that 'bone age' was determined in all the subjects. Radiological examinations of the hand were done using standards developed by Buckler (1977). According to Buckler the bones of the wrist and hand are radiographically most useful for estimating bone ages for the overall age-range of the growing child. In all the children, the appearance of carpals, the heads of the metacarpals, the phalanges, and other ossification centres were recorded.

Appearance of upper ends of radius and ulna was recorded from X-ray films of elbow according to method developed by Gray (1969). Carpal bones were given more importance while determining bone age, as they are considered more sensitive indicator for skeletal development (Todd 1937). The hand showing an advanced development was considered for assessing bone age, in case if an unequal number of carpals appear in both the hands. The angulations of each carpal bone and the epiphyses of the distal part of the radius and ulna were matched with suitable standard roentgenograms for children of each age and sex. If carpal bones showed delayed development, and other epiphyses showed an advance appearance, development and fusion of other epiphyses helped to determine bone age more accurately. Skeletal age determined by above procedure was compared with chronological age. Length and cortical thickness of the second metacarpal bone was also measured.

Results and discussion:

The difference between skeletal age (SA) and chronological age (CA) in boys and girls was grouped into three categories of $SA > CA$, $SA < CA$ and $SA = CA$. It was interesting to note that almost same percentage of children from both sexes (about 20%) fell in the category of $SA = CA$ (Fig.1). Again almost 60% of both the sexes were found to be borderline cases with a difference of less than 12 months in the category of $SA < CA$. When the age was considered it was seen that while about 13% of 6-10 year old boys were categorized as $SA = CA$, only about 6% of the girls fell in this category. Again, it was observed that as the age advanced the percentage of children sliding from the category of $SA > CA$ to the category of $SA < CA$ increased, indicating that the risk of being affected by fluorosis is higher.

TABLE 1. DISTRIBUTION OF DIFFERENCES (MONTHS) BETWEEN SKELETAL AGE (SA) AND CHRONOLOGICAL AGE (CA) IN BOYS AND GIRLS

Age (Yrs)	% with $SA < CA$.			% with $SA = CA$	% with $SA > CA$.		
	>25	13-24	0-12		0-12	13-24	>24
Boys							
6-10	22.0	13.6	14.8	6.2	27.0	13.6	2.5
10-14	10.2	16.0	44.4	12.0	14.0	2.8	0.9
Girls							
6-10	4.2	12.7	18.6	12.7	23.9	18.3	8.5
10-14	10.9	18.5	42.4	8.7	18.5	0.0	1.1

The cortical thickness of the second metacarpal bones in boys and girls in the present study compared well with that reported by Shah (1983) for rural areas near Baroda. It was also observed that the same was slightly higher in girls than boys at all age groups (Table 2).

TABLE 2. CORTICAL THICKNESS OF CHILDREN FROM FLUOROSIS AREAS

Age (Yrs.)	Thickness (mm)	
	Boys	Girls
6-8	2.41 \pm 0.66 (26)	2.67 \pm 0.47 (23)
8-10	2.79 \pm 0.47 (55)	3.09 \pm 0.51 (48)
10-12	3.33 \pm 0.60 (54)	3.40 \pm 0.73 (54)
12-14	3.77 \pm 0.49 (56)	3.80 \pm 0.54 (38)

Values are mean + S.D. with number of subjects in parentheses.

With regard to the length of the metacarpal bone in these children it was interesting to note that the same was slightly better in the girls than in the boys between the ages of 6-10 years. But a reverse order in the same was seen in the age group of 10 to 14 years confirming that the boys outgrow in the teen age (Table 3).

It is not surprising that about 25% higher values were reported for children in Mexican Americans (Garn et al 1976) than those obtained in the present study for all the parameters measured. But surprisingly the values reported for rural children from Gujarat were lower than those in the present study. This only shows that the children from the fluorosis-affected areas are not at any higher risk than the others living in the better environmental conditions. Nevertheless this comparison may not be very valid as the time gap between the earlier studies and the present one is too large. But in the absence of recent data on the children in this regard the available data serves as a directional one.

TABLE 3. LENGTH OF SECOND METACARPAL OF CHILDREN FROM FLUOROSIS AREAS

Age (Yrs.)	Length (mm)	
	Boys	Girls
6-8	38.1 \pm 2.8 (26)	39.2 \pm 9.0 (23)
8-10	41.5 \pm 4.2 (55)	44.1 \pm 5.3 (48)
10-12	51.2 \pm 6.2 (54)	48.3 \pm 8.1 (54)
12-14	57.4 \pm 5.0 (56)	54.9 \pm 4.9 (38)

Values are mean + S.D. with number of subjects in parentheses.

Conclusions:

In spite of the exposure of the children in these affected areas to skeletal fluorosis due to high fluoride intake through water, the risk of these children as measured by the cortical thickness and length of the metacarpal bones as well as the skeletal age in relation to their chronological age, is not seen.

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**ASSESSMENT OF A PATIENT EDUCATION PROGRAMME
FOR ADULT ADVANCED RENAL FAILURE PATIENTS
(PREDIALYSIS AND HAEMODIALYSIS)**

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The current study, which implemented the educational programme developed by Castelino (2001) for advanced renal failure patients and assessed its effectiveness, was prompted by the virtual absence of such research in the Indian context. The sample consisted of 18 (9 each of predialysis and haemodialysis) advanced renal failure patients. The majority had been diagnosed to have Chronic Renal Failure in 2001. An interview schedule consisted two parts (A & B) as the pre-test and post-test measures. A score card was used to assess the impact. The education programme that was conducted on 5 groups of patients (3 predialysis, 2 haemodialysis) extended over three sessions (2½ hours each) for one week. Overall, the post-test score ($M=169.17$) was significantly higher than the pre-test score ($M=81.17$; $t=17.897$; $p=0.000$). Regarding knowledge, the post-test score ($M=113.94$) was significantly higher than the pre-test score ($M=46$; $t=16.92$; $p=0.000$). Regarding adjustment, the post-test score ($M=55.22$) was significantly higher than the pre-test score ($M=35.17$; $t=9.320$; $p=0.000$). These findings attest to the effectiveness of the programme.

Most often kidney failure is permanent and one has to undergo dialysis to clean the blood so that toxins and fluids do not accumulate in the body and cause major problems. The only other alternative is a kidney transplant. As a result of the afflicted person's dependence for life upon dialysis, adverse impacts are created upon his/her emotional well-being, somatic identity, sexuality, occupational identity and social roles (Lambert and Lambert 1979). It is seen that low adherence to the medical regimen, diet, fluid restrictions and dialysis, critical for survival, in advanced renal failure patients (Becker 1978; Czackes and De-Nours 1978).

In view of the repercussions of advanced renal failure and the low level of adherence to the treatment regimen, efforts need to be channelised in the direction of patient education, aimed at providing information about the illness and treatment as well as coping strategies. It has been found that only a few foreign patient education programs have been developed and evaluated for chronic renal failure patients. In the Indian context, only two such programs have been developed; one by Rajapurkar and the other by Castelino. As these programs have not been evaluated, the present study aims at assessing the effectiveness of the patient education programme for advanced renal failure patients developed by Castelino (2001).

Materials and Methods:

The sample consisted of 18 advanced renal failure patients attending a public hospital in Mumbai. Of these 18 patients, 9 were predialysis patients (who had not yet begun dialysis) and 9 were haemodialysis patients (who were undergoing dialysis). Only those patients who (a) attended the King Edward Memorial Hospital (KEM) Parel, Mumbai, (b) were between the ages of 20-50 years, (c) could understand and speak Marathi, (d) had been undergoing dialysis for at least a week (haemodialysis) or were candidates for dialysis, but for whom the need for dialysis initiation was not anticipated for the next 3 months (predialysis) were considered for the study. Patients on peritoneal dialysis and patients who had undergone a transplant were excluded from the study.

Eleven male patients and 7 female patients constituted the sample (mean age 39 years). Most of the patients were Hindus. Thirteen were married, 4 were single and one was widowed. Their educational level ranged from having no education to having a graduate degree, with the majority educated upto the 10th/12th standard. Eight patients were employed. Their monthly income was below Rs 6000. The majority had been diagnosed to have Chronic Renal Failure in 2001.

Measurement:

The study employed a pre-test-post-test design. The pre-test / post-test measure comprised of a series of questions, the responses to which were elicited by an interview. The interview schedule included two aspects, viz. Knowledge (A) and Coping / Adjustment (B). The knowledge aspect, consisted of 24 items (closed ended and open ended) and assessed the information and comprehension of patients in areas such as description of the kidneys, causes and symptoms of kidney failure, haemodialysis and peritoneal dialysis, transplantation, diet, strategies for dealing with life issues. The score ranged from 0-159, with higher scores indicating greater knowledge. (Cronbach's alpha was 0.8819 at pre-test and 0.9393 at post-test).

The coping aspect, consisted of two parts. The first part had 46 items (agree / disagree, comprehension) and assessed the coping of patients in various areas, viz. adjustment to treatment, diet and medication, coping in general health, physical appearance, social, emotional, occupational and financial areas. The total score ranged from 0-68, with higher scores indicating better adjustment (Cronbach's alpha was 0.8186 at pre-test and 0.8061 at post-test). The second part tapped the cost effectiveness of treatment. The Perception of Treatment Benefits and Treatment Barriers Scale which was constructed by Castellino (2001) for End-Stage Renal Disease patients based on the Diabetes Specific Health Beliefs questionnaire developed by Bradley (1993) was employed. It consisted of 12 items, 6 tapping perceived treatment benefits and 6 tapping perceived treatment barriers. The subjects had to rate these items on a 4-point rating scale. A measure of cost effectiveness was obtained by subtracting the barrier score from the benefit score. The score ranged from -18 to +18, with higher positive scores indicating greater perceived benefits (Cronbach's alpha was 0.6459 at pre-test and 0.6178 at post-test). Qualitative analysis was also conducted wherever applicable.

The patient education program which was conducted on 5 groups of patients (3 predialysis; 2 hemodialysis) extended over three sessions (2½ hours each) and concluded within a week. Variety of teaching aids (role play, computer presentation, demonstration, group discussion, homework assignments) were used during the workshop.

Assessment of the instructor is also a very important task in evaluating the programme. Four factors are important in assessing the ability to teach: energy, attitude, knowledge and skill (Cookfair, 1991). The facilitator was well versed with the various topics covered to clarify patient's doubts such as dialysis sessions.

Results and Discussion:

Overall the post-test score ($M=169$) was significantly higher than the pre-test score ($M=81$, $t=18$, $p=0.000$). Regarding the aspect of knowledge, the post-test score ($M=113$) was significantly higher than the pre-test score ($M=46$, $t=17$, $p=0.000$). At this juncture, an examination of the individual items seems relevant. Regarding the items tapping information, an overwhelming majority of patients did possess information, even prior to the program about a few aspects, such as that the kidneys are bean shaped (94%), that an individual has two kidneys (100%), that the

function of the kidneys is to remove wastes, toxins and excess water from the body (94%), that high blood pressure and excessive use of drugs are potential causes of kidney failure (78%), that swelling around the eyes and ankles is one of the symptoms of kidney failure (83%) and that haemodialysis patients need to have an access created prior to dialysis (100%).

However, subsequent to the patient education programme, the patients did gain information on a substantial number of aspects. While fewer knew at pre-test that the kidneys were the size of fist (50%), that they are reddish-brown in colour (39%) and that they are located above the waist on either side of the backbone (50%), all patients had this information at post-test. That hereditary factors and uncontrolled diabetes could be potential causes of kidney failure were known to only 17% and 44% of the patients at pre-test. However, at post-test, many subjects were in possession of this information (67% and 89% respectively).

Interestingly, at pre-test, only one patient knew that a diagnosis of End Stage Renal Disease was made when the kidneys functioned at only 10% of their capacity. At post-test, 56% were armed with this information. Surprisingly, only few patients knew at pre-test, that haemodialysis was the name of the dialysis they were / would be undergoing (2), that transplantation was an alternative treatment option (3) and that blood group matching (4) and tissue type matching (1) were essential criteria for transplantation. At post-test however, 56% could recollect the term 'haemodialysis' and all were equipped with the information that transplantation was an option available to them. A large number also indicated that blood group matching (78%) and tissue type matching (67%) were essential prerequisites for transplantation.

Regarding the critical issue of diet, while more than half (56%) knew at pre-test that sodium had to be restricted, only 33% and 22% respectively were knowledgeable about the restriction of potassium and protein and none were aware about the necessity for phosphorous restriction. At post-test however, 89%, 78%, 78% and 72% were aware of the necessity of restricting sodium, potassium, phosphorous and protein respectively. While only 44% were aware of the fact that fluid restriction was necessary as excessive water accumulates in the body leading to symptoms, like swelling, at pre-test, all were cognizant of this fact at post-test.

With respect to the subject of marriage, only 28% at pre-test understood that patients could get married if they wished to but at post-test, almost all understood this aspect. Again, the need to discuss with the spouse about the illness (nature, consequence) treatment (schedules and costs) and responsibilities of the spouse (preparing a special diet, providing assistance) was realized by a far greater number at post-test than at pre-test.

As is evident from the Table 1, which presents a list of 8 statements, to which the patients had to respond in terms of agree / disagree / don't know, an increase in the percentage of patients stating the accurate response for all the items was noted from pre-test to post-test.

Regarding the items tapping comprehension, a number of open-ended questions were posed. In response to the first question "What would you do if the medicine prescribed was not available but a similar one was suggested by the shopkeeper?" while a majority (83%) rightly stated at pre-test and at post-test that they would consult a doctor, surprisingly, not one responded, even at post-test, with the ideal answer that they would stock up medicines in advance so as to avert an emergency situation from developing. When queried as to what they would do if they were invited to a party and served food they were not permitted to eat, only 22% thought, at pre-test, of eating at home prior to the social function. However, 61% mentioned this response at post-test.

On being questioned as to what they would do if their access (fistula) needles came out during dialysis, although 67% did mention at pre-test that they would call for help from the dialysis staff, none indicated that would hold their hand firmly over the relevant spot. On the other hand at post-test, 67% indicated both the above mentioned aspects. In response to the question what they would do if they had to travel out of Mumbai for some days, only 11% at pre-test but 61% at posttest rightly indicated that they would make enquiries about dialysis facilities and set up appointments with the dialysis unit at the places they intended to travel to.

TABLE 1: ACCURATE RESPONSES (%) TO STATEMENTS

	Statements	Pre-test	Post-test
1	A patient cannot be completely cured in one dialysis session	72.2	100
2	During dialysis, only a small portion of the patient's blood is outside the body	50	100
3	A patient cannot have more than one kidney transplantation	16.6	88.8
4	One can eat whatever one wishes to before dialysis, as the excess will get washed away during dialysis	11.1	14.7
5	When the access itches, becomes red or swells, any soothing ointment can be applied	55.5	94.4
6	Patients can drink fruit juice or coconut water when they feel thirsty	83.3	100
7	A patient cannot travel by public transport such as a bus or a train	44.4	83.3
8	Only one dose of the Hepatitis B vaccine has to be taken	16.6	94.4

N.B: Only statements 1 & 2 are correct statements

With respect to the adjustment aspect, the post-test score ($M=55.2$) was significantly higher than the pre-test score ($M=35.2$; $t=9.3$; $p=0.000$). It is evident from the mean scores in different areas of adjustment (Figure 1), that although in all areas there was an increase in scores from pre-test to post-test, the greatest increase was noted in the emotional area, while the least was observed in the area of general health and physical appearance.

Finally, addressing the aspect of treatment benefits and barriers, the cost effectiveness at pre-test was 6.83 while at post-test it was 10.8. Thus, at post-test, patients perceived treatment benefits to a greater degree than at pre-test. With respect to the individual items, it was noted that at post-test, patients perceived to greater extent the relevance of regular exercise, taking dialysis on time, caring effectively for their access and controlling food and fluid intake. They also perceived, to a lesser extent, the restrictions imposed by the illness on food and fluid intake and by dialysis on job performance and social-recreational activities and the difficulties involved in taking medicines and caring for their access.

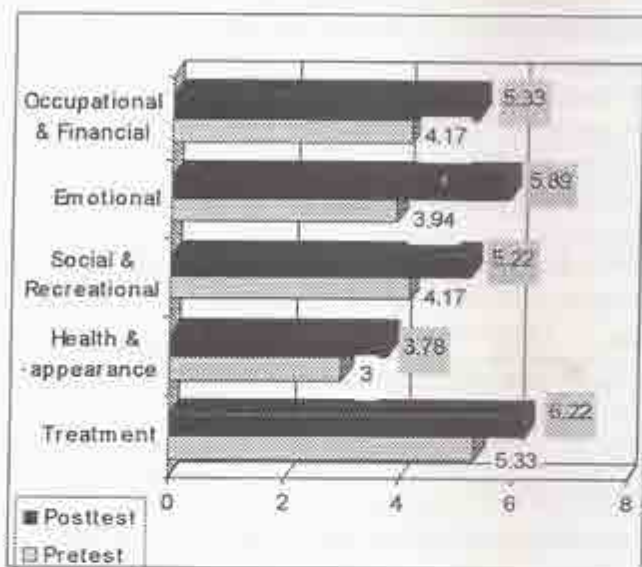


Fig.1. Mean scores in different areas of adjustment

An examination of the individual items indicated, that at pre-test itself, the patients did have information about the number and shape of the kidneys, the excretory function of the kidneys, two potential causes of kidney failure (high blood pressure and excessive use of medication) and a symptom of kidney failure (swelling). Patients had probably seen books, pamphlets and signposts in the nephrology department depicting the number and shape of the kidneys. Some of them may have also been informed by the doctors that they were undergoing dialysis or may have to undergo dialysis because both their kidneys were damaged. An experience/observation of dialysis being instrumental in the removal of wastes could have enabled the patients to realize the excretory function of the kidney. Many lay people are of the opinion that excessive medication can cause harm to the body and hence patients may have generalized this belief to the kidney as well. Since many patients had a history of high blood pressure and experienced swelling, they could identify high blood pressure as a cause and swelling as a symptom of kidney failure.

For a large majority of items however, the programme proved to be highly beneficial. Patients were helped to identify the colour of the kidneys by pointing out the similarity between the colour of "rajma beans" and that of the kidney. To help them understand the concept of kidney failure, which only one patient knew at pre-test, the researcher used an analogy. She compared the fully functioning kidney to the value of 100 rupees and the loss of 90 rupees with consequently only 10 rupees remaining, to 10% of kidney functioning, which indicates kidney failure. Another function of the kidney, namely, the stimulation of the production of red blood cells was explained by bringing out the role of erythropoietin and its function of producing red blood cells in the bone marrow. This was also elucidated with the help of an analogy of a postmaster instructing the postmen to deliver letters to the various households.

Only 2 patients knew that the dialysis they were undergoing/had to undergo in the near future was "haemodialysis". It is essential for patients to know the specific name of their treatment or else there are chances of miscommunication which in turn could have an adverse effect on them.

The programme helped patients become aware of the name of their treatment and also the fact that kidney transplantation was an alternative treatment option which only one patient was knowledgeable about. Perhaps, newly diagnosed patients did not have an opportunity to obtain this latter information prior to the programme and the others may not have been in a frame of mind to register this information, as they were probably still in the process of adjusting to the fact that they had kidney failure.

Knowing the reason for restricting certain food elements can help patients feel in control of their illness and treatment and reduces dependency on the doctors/dieticians for every decision pertaining to the diet regimen. During the programme, patients were instructed as to which food elements they needed to restrict (sodium, phosphorous, potassium & protein). They were also helped to identify the food element present in the restricted foods (protein in milk and cheese) and the reason for not consuming /restricting certain food items (restriction of milk because it is high in protein and the body is not able to remove excess proteins, resulting in an accumulation of proteins and a build up of toxins).

The programme was successful in eradicating erroneous ideas. For example, the belief that one could not go in for a second transplant was dispelled by helping patients realize that one failed transplant does not preclude the next transplant and that one could go in for another transplant, provided another donor was available. The myth about being able to eat any food prior to dialysis, as the excess would be washed away during dialysis was disputed by pointing out to the fact that this could result in toxic substances building up in the body and reaching alarming levels, before the scheduled dialysis day. The idea that they need only one dose of the Hepatitis B vaccine was rectified by explaining to them that they require multiple doses for efficacy. Patients were particularly encouraged to discuss issues of marriage, sexuality and childbirth with the doctors and to confide in the spouse the difficulties / implications of each of them.

Regarding the adjustment schedule, the greater reported adjustment was in the emotional area and the least in the area of general health and physical appearance. During the programme, the importance of communicating one's needs to family members was brought up. Also, methods of reducing one's negative thoughts and beliefs and of relaxation were taught to the patients. This could have accounted for the increase in scores in the emotional area. On the other hand, the meagre increase in scores in the area of general health and physical appearance could be explained by the fact that the toxic effects of uraemia are manifested in virtually every body system which is bound to affect general health.

Regarding the cost-effectiveness of treatment, the patients perceived greater treatment benefits than barriers at pre-test itself and even greater treatment benefits at post-test. This finding suggests that such a healthy and positive attitude could certainly influence behaviour and consequently better adjustment.

Some additional observations made about the programme were that male and female patients were comfortable in each other's presence. There was a feeling of camaraderie among the patients. They often reassured fellow patients who were anxious and helped them understand various concepts. Although haemodialysis patients had somewhat greater knowledge than their predialysis counterparts in some issues related to illness and treatment, they were not more knowledgeable in other aspects. Patients did have a lot of queries pertaining to diet and although the researcher did her best to handle the questions, the need of a dietician was felt.

Conclusion:

The effectiveness of the package has demonstrated that regardless of the literacy level, all patients have the potential to gain information and acquire coping strategies. Indeed, patient education packages are particularly valuable for the lower socio-economic groups, who may have minimal access to / or may not benefit from other sources of information such as books, journals and the Internet. Such programmes should be conducted in a group context as peer support and tutoring were found to be powerful propellers of change. Mixed groups consisting of both predialysis and haemodialysis patients can help patients benefit from each other. Role-plays in particular were found to be appealing and effective with the target group.

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ASSESSMENT OF PHYSICAL FITNESS OF WOMEN IN AGRICULTURE

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The present study assessed the fitness of the women in agriculture. The sample consisted of randomly selected thirty rural women between the ages of 25-45 years. The physical fitness was determined by two different methods i.e. estimation of aerobic capacity and step test ergometer. The results revealed that majority of the respondents belonged to high average fitness level on the basis of aerobic capacity. But according to the exercise on step test ergometer, they had poor physical fitness suggesting the need for better nutrition.

Agriculture being the main occupation of rural India the contribution of women to the agricultural field is estimated to be 70- 80 per cent, mainly as the labour force for all the agricultural activities. For better productivity, women should be healthy and have good physical fitness that refers to the physiological fitness or the cardio respiratory fitness as determined by the maximum aerobic power (VO₂ Max) of an individual (Chauhan 1999). Systematic research on the fitness of women in agriculture is scanty. An effort is being made to study the physical characteristics and physical fitness of rural women actively engaged in agricultural activities under All India Coordinated Research Project, Department of Family Resource Management, University of Agricultural Sciences, Dharwad, Karnataka.

Materials and Methods:

Based on the active involvement of the women in all the agricultural activities thirty women between the ages of 25-45 years were randomly selected for the study. Care was taken to select normal women who are non-pregnant, non-lactating and with no major cardio vascular diseases or any handicap.

The physical measurements such as weight, height, body fat and grip strength were made for all the women. The physical fitness was determined by two different methods: Based on their height and weight the consumption of maximum volume of oxygen (the aerobic capacity (VO₂ Max) of the respondents was estimated. The respondents were grouped according to the classification given by Saha (1999) (Table 1).

$$\text{VO}_2 \text{ Max (l / min)} = 0.023 \times \text{body weight (kg)} + 0.034 \times \text{age (yrs)} + 1.652.$$

$$\text{VO}_2 \text{ Max (ml / kg / min)} = \text{VO}_2 \text{ max (l / min)} / \text{body weight} \times 1000.$$

Physical fitness was determined by using the step test ergometer and the heart rate monitor. The heart rate monitor was fitted to the selected respondents and two minutes relaxation time was given to adjust to the heart monitor. Then the monitor was switched on to record the heart rate. The subjects were made to sit in a relaxed position for five minutes to get the resting heart rate data. From sixth minute onwards they were asked to perform the step test exercise on the step stool measuring 29.0 cms breadth, 45 cms length and 24 cms height till they get exhausted or up to five minutes, at the rate of 30 steps per minute. Immediately after termination of the exercise the subjects were given rest in sitting position for five minutes for recovery. The heart rate was recorded continuously for the rest, exercise and recovery periods. Then the physical

fitness index score was determined by using the following formula: $PFI = \text{Duration of stepping (in sec)} \div \text{Sum of recovery heart rate at 1, 2 \& 3 minute multiplied by 100}$.

The respondents were grouped as per the classification score given by Varghese et al (1994). Based on the Body mass index (BMI) of the women calculated by using the Quetelets Body Mass Index formula the respondents were classified according to Garrow (1987).

TABLE 1. CLASSIFICATION RANGE OF PHYSICAL FITNESS LEVELS

Category (ml /Kg / Min)	VO2 Max	PFI Score
Poor	≤ 15.0	< 80
< Average	15.1 - 22.5	81 - 100
> Average	22.6 - 30.0	101 - 115
Good	30.1 - 37.5	116 - 135
Very Good	37.6 - 45.0	136 - 150
Excellent	> 45.0	> 150

Results and Discussion:

The mean age of the respondents was 35.6 years with the SD of ± 7.57 , mean height was 150.9 cm and the mean weight was 45.4 kg. The body mass index depicted that most of the respondents belonged to low weight range with a mean score of 19.9 per cent. The mean aerobic capacity was 33.3 ml / kg / min of VO2 Max. The mean blood pressure of the respondents fell in the normal range i.e. 115 / 69 with pulse rate of 75 beats / min. The mean body temperature was 97.3o F. The average grip strength of right and left hand of the respondents was 20.6 kg and 19.5 kg respectively.

Highly significant positive correlation was observed between body mass index and VO2 Max of the respondents while highly significant negative correlation was observed between the weight and VO2 Max. Majority of the respondents (43.3%) belonged to the normal body mass index range followed by 26.7 per cent in the low weight range. None of the respondents belonged to the obese range of both the grades.

All the respondents fell in the above average to excellent category of physical fitness according to their aerobic capacity. On the other hand, exercise on step test ergometer and the heart rates revealed that a maximum of 40 per cent of the respondents fell in the poor physical fitness level followed by 33.3 per cent in the high average category (Fig 1).

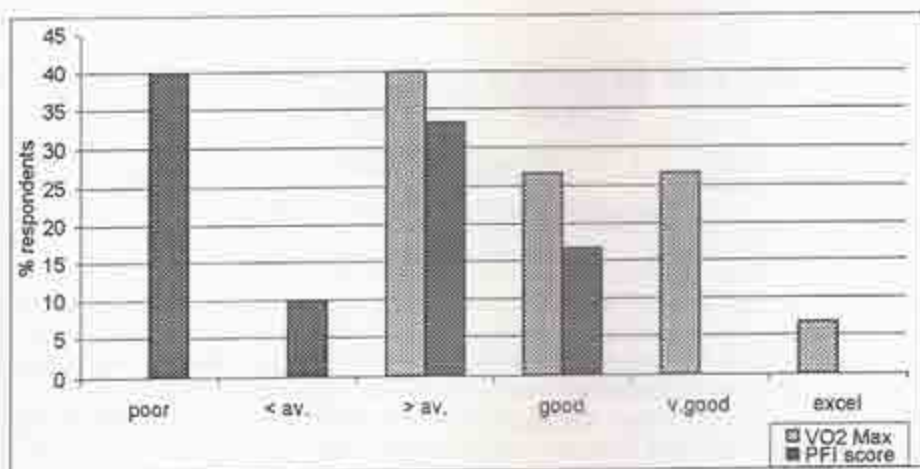


Fig. 1. Physical fitness level by Aerobic capacity (VO2Max) and Step test (PFI score).

It is evident from the above graph that the assessment by VO2Max slides towards the excellence in physical fitness while the PFI scores indicates a reverse trend. This only means that there is no correlation between the two methods. Thus the need for validation of the methods for the sensitivity to test the physical fitness level is felt. Interestingly, further enquiry on the perceived exertion of the step test revealed that all the respondents with no exception found the test to be very exerting after the exercise.

Conclusion:

As the two methods varied widely with the assessment of physical fitness it was difficult to judge the fitness status of the women engaged in agricultural activities.

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IMPACT OF MOTHER'S EDUCATION ON NUTRIENT INTAKE OF TEENAGERS

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Food and nutrient intake of 137 boys and 128 girls, aging between 13 to 17 years, studying in 8th to 11th standards in three schools of Rajkot, catering to different economic levels were measured through 3 days dietary intakes. Nutritive value was calculated using the food tables. Results showed that, mean nutrient intake of subjects of well-educated mothers was higher than less educated mothers, but deficits in iron, carotene and free folic acid were observed in both the groups. Mean nutrient intake was lowest in girls of less educated mothers.

It is well established that the teen-agers want to be independent and long for an identity of their own. In the quest of this they revolt / repel parental suggestions and skip breakfast or refuse to eat certain foods. The values and opinions of the peer group exert a great influence on the adolescents' food selections. Thus there are a variety of factors that may influence the food intake of teenagers. Fathers' occupation and fathers' educational levels showed very little or no differences in the dietary intakes of children of 11, 13, and 15 years age in Australia (Magarey, 1997). As in India the mothers have more influence on the food intake of the family members, it was decided to study the influence of the mother's education on the food and nutrient intake of teenagers studying in 8th to 11th standard, ageing 13 to 17 years.

Materials and Methods:

In all 137 boys and 128 girls in the age group of 13 to 17 years studying in 8th to 11th classes were selected for this study from different schools of Rajkot City of Gujarat state. Subjects were selected randomly from high schools based on their income group i.e lower income group (LIG), middle-income group (MIG), and higher income group (HIG) for each standard. A questionnaire was designed to collect information on 24-hour dietary intakes for three days, including a holiday-Sunday. Nutritive value was calculated using food tables (Gopalan et al 1996).

Results and discussion:

It was observed that, almost equal number of female and male subjects were from LIG (44 & 52 %); MIG - 36 & 32 %) and HIG - (48 & 53 %) in the subjects selected. While in LIG and HIG majority of the parents had an educational of only up to school level, quite a few of the parents in MIG were graduates. As expected the mothers' educational level was lower than the fathers' in MIG (42 and 47 % respectively) but larger number of mothers in HIG were graduates as compared to fathers.

TABLE 1. MEAN FOOD INTAKE BY TEENAGERS OF WELL-EDUCATED AND LESS EDUCATED MOTHERS

Food Group	Graduation and above		School and less	
	Boys N = 44	Girls N = 41	Boys N = 80	Girls N = 72
Cereals and Millets (g.)	282	229	254	208
Pulses and Legumes (g.)	49	51	47	44
Leafy Vegetables (g.)	25	24	19	21
Roots and Tubers (g.)	96	68	84	75
Other Vegetables (g.)	41	40	45	35
Nuts and oil seeds (g.)	2	4	4	3
Fruits (g.)	72	67	47	54
Milk & Milk Products (g.)	461	480	475	443
Fats and oils (g.)	47	37	38	33
Sugar and Jeggery (g.)	35	30	33	31

The food consumption pattern did not differ between the two groups with different educational levels although a small difference in the amount of cereals and millets and leafy vegetables were found to be higher in children of educated mothers. Education did have a significant influence on the intake of fruits as well as fats and oils among the teenagers.

Boys consumed more of all the food groups than the girls in general. Consumption of leafy and other vegetables was low in all the groups. Thus the educational level of the mothers did not seem to influence the food intake except for fruits. As income of the family is closely related to the education in our country, the difference in fruit consumption in the two groups could be due to the purchasing capacity and prioritization of the food groups to suit the pocket.

The nutrient intake as calculated from the consumption of different food groups was compared with the RDA for this age group and it was found that the mean intake of Calories, protein, iron, riboflavin, folic acid were deficient in teenagers of both the educational levels but was to a much greater extent in the less educated group.

Again there was a significant difference between the boys and the girls. Mean nutrient intake was lowest in girls of less educated mothers. The consumption of nutrient dense foods was also observed to be low in the teenagers whose mothers were less educated. Thus, the food and nutrient intake is greatly influenced by the sex and the family income, which depends on the educational level of the breadwinners.

TABLE 4: MEAN NUTRIENT INTAKE OF CHILDREN OF WELL EDUCATED AND LESS EDUCATED MOTHERS

Nutrient		Graduation and above		School and less	
		Boys N=44	Girls N=41	Boys N=80	Girls N=72
Protein	g.	61	54	55	48
Fat	g.	75	64	65	57
Fiber	g.	7	5	6	5
Carbohydrates	g.	309	262	276	246
Energy	Kcal	2211	1891	1955	1747
Minerals	g.	12	10	11	10
Calcium	mg.	911	845	814	809
Phosphorus	mg.	1520	1323	1403	1212
Iron	mg.	16	14	15	13
Carotene	mg.	861	907	787	697
Thiamin	mg.	1.5	1.2	1.4	1.0
Riboflavin	mg.	0.9	0.8	0.8	0.7
Niacin	mg.	13	11	13	10
Folic Acid Free	mg.	60	50	57	47
Folic acid Total	mg.	181	157	174	142
Vitamin C	mg.	65	56	54	53
Choline	mg.	255	231	184	179

Conclusion:

It has been found that educated mothers provide better nutrient dense diets to their children. Although per capita income may also have played a great role as the educational level and the income are inter related in our country.

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BIOTECHNOLOGY- GREEN REVOLUTION IN TEXTILE INDUSTRY*Inderpal Rai*

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Biotechnology can most easily be defined as any technology that relies on living organisms or biological systems. Human beings have been using biotechnology for thousands of years to produce food products, textiles and other necessary items. Some of the familiar items include leavened (yeast-rising) bread, yogurt, cheese, wine, beer and vinegar. All of these products are produced with the help of cultured microorganisms. The term "biotechnology" has come to mean the use of genetic engineering, and associated techniques, in a variety of applications from medicine, textile to agriculture. Although biological systems offer many attractive possibilities and new approaches to all sorts of problems and needs, there is still a great concern in the society.

Biotechnology is the process by which natural components of the body are produced in sufficient quantities to use therapeutically. The pharmaceuticals produced in this way are virtually identical to the naturally occurring materials. These products are usually proteins and have a very specific physiological role, and as a result they may have fewer undesirable side effects associated with them. By contrast, traditional drugs are produced through synthetic organic chemistry and are often less specific in their activity. This can result in numerous side effects limiting the utility of the drug.

To produce these products, scientists use the techniques of genetic engineering to introduce into bacteria, yeast or cultured animal cells the information needed to produce a human protein that has therapeutic potential. Once engineered, these cells can be grown in large quantities often using the time-honored technique of fermentation.

Textile Applications :

Cellulase "stone-washing" : The most widely used application of cellulases (neutral pH cellulases) is the replacement of pumice stones in the "stone-washing" process to produce the aged appearance of denim garments. Using cellulases in replacement of pumice stones prevents damage by abrasion to washing machines and the garments, eliminates the need for disposal of the used stones and improves the quality of the wastewater. The load of garments may also be increased by as much as 50 %, since stones are no longer added. Depending on the finishing effect required, a mixture of cellulases and pumice may be used. Washing the garments with pumice stone removes the surface dye by abrasion, reducing fabric strength. A controlled treatment with cellulases, however, hydrolyze primarily the surface of the fiber but leaves its interior intact. This mode of action makes cellulases suitable for the "stone-washing" of garments dyed with indigo, since this dye stays on the surface of the fiber. Cellulases produced by *Humicola insolens* have usually been preferred for cellulase "stone-washing" because they are neutral cellulases with greater EG activities than acid cellulases (e.g. those produced by *Trichoderma reesei* fungi). It is already recognized that endo activity (EG) play a major role among the stonewashing cellulases; greater EG activities achieve the desired aged look with lower levels of indigo back staining (redeposition of dye in the garment). This effect is best obtained using vigorous mechanical

action (Cavaco-Paulo et al 1998). Genencor Inc. claims that the use of proteases together with cellulases reduced the back staining even further, so that acid cellulases may be used in stonewashing.

Finishing processes

The 'Biopolishing' process, involves the use of an enzyme to improve the handle and appearance of fabrics. 'Biopolishing' is an enzyme treatment designed to improve fabric quality. This treatment offers the following advantages: Durability and maintenance of quality even after repeated wear and laundering & improved pilling resistance. Pilling consists of fluffy agglomerations of loosened fuzz attached to the surface of the fabric. Pilling may occur during fabric processing, as well as during garment wearing and laundering. Biopolishing of cellulosic fabrics, such as cotton, linen, rayon and Lyocell involves treatment with a cellulase enzyme, which weakens the ends of the fibers protruding from the fabric surface. Subsequent gentle mechanical action breaks off the weakened ends from the body of the fabric.

Cellulases are used in Biopolishing, improving the appearance of cotton fabrics by removing fuzz fiber and pills from the surface, which reduces pilling propensity, and improves handle by increasing fabric flexibility and decreasing fabric stiffness.

There is also an increasing use of cellulases in domestic washing products, where they are claimed to aid detergency and to remove damaged fibril material, improving fabric appearance, softness and color brightness. Cellulases are frequently used together with proteases and lipases. Proteases help removing protein based stains (such as egg and blood) from clothing, while lipases are responsible for removing fatty substances.

Cellulases are also being used to replace singeing (a rather hazardous physical process), which is applied to many cotton goods prior to scouring and bleaching. The fabric is passed between flames to burn off the lint and threads that extend beyond the surface of the material to give a smooth surface to the fabric.

Pectinases have been used in the treatment (retting) of jute and flax to separate the fibers and eliminate pectin. Recently, it was found that the treatment of jute, flax and ramie with cellulases improves the mechanical properties of the fibers, increasing its flexibility with good retention of tensile strength.

A treatment with a mixture of cellulases and xylanases prior to peroxide bleaching was found to enhance significantly the brightness of jute fibers, reducing therefore the peroxide requirement for bleaching. This treatment was also found to produce softening benefits.

Felting of wool fibers is primarily caused by the presence of scales at the fiber's surface. This can be prevented to an extent using oxidizing and reducing agents, which are pollutants. The use of proteases has recently been investigated, but the results obtained so far present a high variance and are therefore not yet used at an industrial scale.

The use of proteases to reduce prickles in wool has been investigated with encouraging results. Proteases have also been used with success together with hydrogen peroxide for wool bleaching.

Dyeing Processes :

Fungi can be used in new production processes that are themselves less polluting than traditional chemical processes and some species of white rot fungi are already being employed to degrade toxic wastes. Many of these applications are still under development. For example, textile dyes could be produced by fermentation in the future (note: before the invention of synthetic dyes in the nineteenth century the colors used to dye textiles came from natural sources such as plants and lichens). Many fungi produce pigments during their growth, which are substantive as indicated by the permanent staining that is often associated with mildew growth on textiles and plastics. Some fungal pigments have been shown to be anthraquinone derivatives, resembling the important group of vat dyes. Fungi therefore have potential as agents for the direct production of textile dyes or dye intermediates replacing chemical synthesis, which has inherent waste disposal problems. The production and evaluation of microbial pigments as textile colorants is currently being investigated at BTG (British Textile Technology Group, Manchester, UK) and preliminary results have been published (Hobson and Wales 1998).

Fermentation is the degradation of complex organic compounds, usually carbohydrates, by enzymes produced by such microorganisms as actinomycetes, bacteria, yeast, and molds. The two major types are aerobic (in the presence of oxygen) and anaerobic (in the absence of oxygen). The process is affected by such other factors as pH, time span of fermentation, and additional ingredients. Equipment should be inert to the chemicals present in the dyestuffs as well as the microorganism that is active in fermentation and any byproducts of the degradation. Fermentation can start fairly quickly and may produce a profound or mild effect on the color from the vat. Any vat that has rested for a few hours or overnight at room or slightly higher temperature has probably begun to ferment (Kadolph 2002).

The use of sodium dithionite (also known as "hydro") for the reduction of indigo has been ubiquitous for a century. A recent study which re-examined the older fermentation methods for indigo reduction using woad leaves, bran, and wood ash suggests that a strain of *Clostridium* bacteria were involved in this vat. A modern biologically based system may offer a better method for the 21st century, and some indigo dyers have expressed interest in the possibilities of using the method. (Anon 1998).

Detergents

Surfactants are widely used in the textile industry, and make a distinct contribution to the BOD and COD loading of textile effluent. Non-degradable surfactants may have low BOD, but they persist beyond treatment, and a degradable surfactant is preferable. A range of highly biodegradable surfactants for the textile industry has been developed. It is claimed that these materials are as effective as the traditional products they are designed to replace in the whole range of textile processes such as wool milling, wool carbonizing, scouring of cellulose, soaping after reactive dyeing, and wetting.

Enzyme processing continues to suggest environmentally friendly opportunities for textile processing. Recent research suggests that bioscouring using pectinase may be carried out even more efficiently in nonaqueous media, the process being quicker and requiring fewer enzymes.

Green technology:

The EPA has released for public comment a draft list of persistent, bio-accumulative and toxic (PBT) chemicals that may occur in industrial hazardous wastes regulated under the Resource

Conservation and Recovery Act (RCRA). In line with the agency's stated preference for pollution prevention (P2) over treatment, these chemicals will be the focus of source reduction and recycling activities. Included on the list are a number of chemicals with textile connections, including several chlorinated solvents, dye intermediate precursors, 1,2,4-trichlorobenzene, chromium, and copper.

Antimicrobials are included in many different products, including a range of textile materials. A recent article describes the EPA's draft to clarify the Treated Articles Exemption (in 49 CFR section 152.25) to Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) regulations for products with antimicrobial claims. The exemption applies to cases where the antimicrobial protects the product rather than the person using the product. The draft is being studied by industry. It requires that control of microorganisms be demonstrated through tests that simulate conditions of use, and that companies must have this test data on file. Several factors can influence the choice of test, but included in the choice are AATCC Test Methods 100 (quantitative) and 30 and 147 (qualitative). One recently developed use of antimicrobial technology involves the use of fibers containing a fungicide that will kill the fungus that dust mites rely upon to break down human skin cells to a digestible form. This will thus eliminate dust mite fecal matter, which is a serious allergen. The fiber was developed by Courtaulds and is called Amicor Plus.

An industrial biopulping/biobleaching process would eliminate the pollution problems associated with the use of chemicals. Lignin-degrading fungi or their enzymes also have the ability to degrade highly toxic organic compounds such as dioxins and PCB's (polychlorinated biphenyls), and could have an important role to play in the remediation of contaminated soils and the disposal of chemical wastes. A recent report also indicates that lignin-degrading fungi can even degrade synthetic textile polymers such as nylon previously thought to be non-biodegradable (Deguchi et al 1997).

The use of fungi as biocontrol agents to kill insects (mycoinsecticides) and weeds (mycoherbicides) has the potential to replace many of the toxic chemicals currently in use. Several species of fungi have now been commercially formulated as mycoinsecticides (Note: spores of *Metarhizium anisopliae* were first used in Russia in the late 1800s as a mycoinsecticide).

Caring for the Environment - Waste Management :

Natural and enhanced microbial processes have been used for many years to treat waste materials and effluent streams from the textile industry. Conventional activated sludge and other systems are generally well able to meet BOD and related discharge limits on most cases.

However, the industry does face some specific problems, which are both pressing and intractable. They include color removal from dyestuff effluent and the handling of toxic wastes including PCPs, insecticides and heavy metals. Not only are these difficult to remove by conventional biological or chemical treatment but also they are prone to 'poison' the very systems used to treat them. The microbes employed need to be versatile and robust towards complex and often varying environments.

Colour Removal :

One of the more pressing environmental problems that have been facing the textile industry is the removal of color from dye bath effluent prior to discharge to local sewage treatment facilities or adjoining watercourses. Brightly colored water-soluble reactive dyes and acid dyes are particularly problematic, passing unaffected through conventional treatment systems at the sewage

works and entering watercourses. Considerable effort has been spent on developing suitable treatment systems for these effluents. Only biotechnological solutions can offer complete destruction of the dyestuff, with a coreduction in biological oxygen demand (BOD) and chemical oxygen demand (COD). In addition, the biotechnological approach makes efficient use of the limited development space available in many traditional dye house sites. (Willmott et al (1998)

Reactive dyes are particularly difficult to treat by conventional methods because they are not readily adsorbed onto the activated sludge biomass where they could be degraded. Zeneca Environet is currently pioneering one approach to this problem, which involves direct microbial attack on the azo-linkage of organic dyestuffs, leading to their complete degradation in solution. Pilot units are already running in a couple of major UK dye houses.

Alternative approaches being evaluated in the UK include the use of biologically active materials such as chitin to absorb color. Researchers in some developing countries are experimenting with more readily available and cheaper local sources of biomass such as straw pulp and even residues from biogas reactors.

Metal and Toxin Removal :

The potential for using selected fungi to absorb heavy metals from effluent streams has already been touched upon. Species such as the ligninase-producing white wood rot fungus have already been successfully applied in the paper and pulp industries for removing lignin-bound chlorine. They are also effective against biphenyls, aromatic hydrocarbons and chlorinated compounds such as PCPs and DDT. Other fungi have been used to remove highly toxic tannins from tannery effluents.

Textile Supports :

A novel approach to promoting aerobic degradation in contaminated lagoons and preventing the development of malodorous and unpleasant anaerobic processes has been pioneered in Germany. Here Hoechst as a support for the microorganisms has patented a development based on a 3-D 'biomat' of knitted polyester monofilament. The mat is stable and resistant to compression; its open supporting structure counteracts the build-up of anaerobic sludges on the bottom of the lagoon. Byrne (1996).

Conclusion:

According to Byrne there is also still great concern in society about the unbridled advance of biotechnology, especially with regard to the modification of natural species with possible unknown long-term consequences. This note of caution needs to be echoed across the whole spectrum of biotechnology developments. Although biological systems offer many attractive possibilities and new approaches to all sorts of problems and needs, considerable advances are still being made in 'conventional' technologies such as catalysis, chemical synthesis and physical fiber modification, which need to be kept in perspective..

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