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Diminishing effect of arjuna tree (*Terminalia arjuna*) bark on the lipid and oxidative stress status of high fat high cholesterol fed rats and development of certain dietary recipes containing the tree bark for human consumption

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It's a fast changing world of glaring contrasts where development and environmental degradation, overfeeding and starvation, occurrence of infectious and life style diseases, take place side by side. The modern medicinal system too, is not exception, curing on one hand and causing sickness through side effects on the other. Heartrendingly, the jolted human discretion has started taking stock of the situation turning to the natural and the indigenous. Ayurveda, the ancient Indian system of medicine is abound with information regarding plant parts and products having medicinal properties sans side effects. Arjuna tree's various parts, especially the bark, occupy the pride of place in the context of such medicinal values. This study has been undertaken to scientifically evaluate theses effects, specially the biochemical and nutritional ones. The study encompassed two phases; the first one being the study of modulating effect of arjuna bark powder on lipid and oxidative stress status of albino rats maintained on high fat and high cholesterol diet; and the second one involving the making of food products incorporating arjuna bark as one of the ingredients. Results of the present study indicate that arjuna bark acts as a hypolipidemic, hypocholesterolemic and oxidative stress lowering agent. Certain recipes incorporating arjuna bark recorded good acceptability meriting their inclusion in the daily diet of the people needing long term intervention for elevated lipids, cholesterol and oxidative stress levels.

Keywords: *Terminalia arjuna*, oxidative stress, hypocholesterolemia, hyperlipidemia

Introduction

The body of scientific knowledge about the role of plant herb and spice in promoting human health and preventing diseases is scanty (Dwivedi, 1997). Though chemical principles present in them have been increasingly researched yet the real worth of this natural wealth remains to be realized. In this context, *Terminalia arjuna* is distributed throughout the greater part of India, Burma and Sri Lanka. It is practically found in the sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh and Deccan regions. Bark of *Terminalia arjuna* has been used in Indian system of medicine for the cure of number of diseases from thousands of years. Ayurvedic physicians recommend the use of *Terminalia arjuna* in the treatment of three types of tumors Vata, Pitta and Kapha (Tripathi and Singh, 1996; Dwivedi and Udupa, 1989; King *et al*., 1954; Cooper, 2005). Arjuna tree's bark, as per Ayurvedic wisdom, reduces body fat and cures

blood and respiratory disorders. Since Ayurveda has been the science of life as a whole , and its percepts have governed to a greater or lesser degree the choice and style of foods in India for millennia, the scientific and nutritional exploration of the medicinal effects as referred to in this indigenous medical sciences could bring immense health benefits. Thus the need to test the curative potential of Arjuna bark, as enunciated by Ayurvedic seers, against the touchstone of 'Scientific Method' prompted this.

Fig.1: ***Terminalia Arjuna*** **plant and bark**

Materials and methods

The study sought to develop experimental models (albino rats) by giving diets containing bark of Arjuna, in addition to the usual ingredients of the normal rat feed and thereafter studying the medicinal/therapeutic effects of the bark on the nutritional/metabolic aspects of the rat tissues.

In the follow up exercise on food product development/improvisation, an endeavor has been made to evolve the powdered bark or its refined form as one of the micro ingredients of the recipe akin to any plant bark based spice.

The attempt also extended to standardization of the contemplated Arjuna bark recipes for its optimum usage to deliver in term of both taste and medicinal characteristics on a long term basis. In an effort to synergistically enhance its prospective medicinal importance, like pickles of lemon as well as ingredients of ayurvedic concoction known as 'Trifala'.

Chemicals: All chemicals used in the study of analytical grade, procured from Credible Concerts in India. Chemical of higher purity and scarcer availability were obtained from M/S Sigma Chemicals Company, St Louis. Some of the dietary ingredients were obtained from ICNP Pharmaceuticals, Incorporation, Life Science Group, Clevelend, Ohio, USA.

Plant material: Bark of Arjuna tree was collected from a village located in Kangra region of Himachal Pradesh after due validation by the corresponding author.

Preparation of diet: Given hereunder is the composition of diet.

Experimental protocol: Twenty male wistar albino rats (100-110g) were randomly divided into four equal groups, consisting of 5 animals each. The groups A, B, C and D received the diets, A

(isoenergic normal fat), B (high fat high cholesterol), C (High fat high cholesterol Arjuna Bark I) and D (High fat high cholesterol Arjuna Bark II) respectively. Diets C and D were prepared by mixing Arjuna bark powder at 5 g/Kg and 10g/Kg respectively to the diet B. Composition of each diet has been given in Table 1. The animals were housed individually in polypropylene cages in a room with about twelve hour light dark cycle, 20-25°C temperature, 40-50% humidity level, and had free access to food and water. Diet consumed by each animal and animals weights were recorded daily.

Table 1. Composition (g/Kg) of normal, high fat and high fat high antioxidant diets

Component	Isoenergic normal fat cholesterol	High fat, high cholesterol	High fat, high cholesterol Arjuna bark I	High fat, high cholesterol Arjuna bark II
	A	B	C	D
Casein (Vitamin and fat free)	220.0	220.0	220.0	220.0
DL-Methionine	2.0	2.0	2.0	2.0
Mineral Mix$_1$	45.0	45.0	45.0	45.0
Vitamin Mix$_2$	10.0	10.0	10.0	10.0
Cellulose	20.0	145.0	145.0	145.0
Sucrose	75.0	50.0	50.0	50.0
Corn Starch	578.0	378.0	378.0	378.0
Oil$_3$	50.0	150.0	150.0	150.0
Cholesterol	0.5	5.0	5.0	5.0
Arjuna bark powder	-	-	5.0	10.0

A. Mineral mixture provided the following g/Kg of all diets CaHPO4, 2H2O, 17.1; K2HPO4, 10.8; CaCO3, 8.1; NaCl, 3.1; Mg O, 0.90; MgSO4.7H2O, 4.0; FeSO4.H2O, 0.39; ZnSO4.H2O, 0.22; MnSO4, H2O, 0.22; CuSO4.5H2O, 0.046; NaF, 0.046; Al2 (SO4)3, K2SO4 0.009; Kl, 0.0036; CaCO3, 0.0036; Na2SeO3. 5H2O, 0.0004.
B. Vitamin mixture provided the following (per Kg of diet): αtocopherol, 50 mg; L-ascorbic acid 0.05g; choline chloride; 0.75g; D-calcium pantothenate, 30 mg; inositol, 50 mg; menadione, 22 mg; niacin, 45 mg; p-aminobenzoic acid, 50 mg; pyridoxine. HCl, 10 mg; riboflavin, 10 mg; thiamine. HCl, 10 mg; retinyl acetae, 9 mg; ergocalciferol, 0.0025 mg; biotin, 0.2 mg; folic acid, 0.9 mg; vitamin B12, 0.013 mg.
C. All the dietary formulations contained blends of corn, groundnut, rapeseed and sesame oils in equal proportion by weight.

At the end of 35 days stipulated time, food was withheld from the rats the previous night and the following morning blood sample collected from orbital plexus of each animal using mild ether anesthesia. Thereafter, the animal was sacrificed by cervical dislocation. The liver was quickly removed, washed with ice cold saline, freed of adhering connective tissue, weighed and a small part used for instant biochemical investigations. The rest of the hepatic tissue was stored at -25° C for further biochemical analysis.

Biochemical analysis: Biochemical analysis carried out included the estimation of serum triglycerides (Foster and Dunn, 1973), serum & liver cholesterol (Zlatkis *et al*., 1953) and lipid per-oxidation (Ohkawa *et al*., 1979) and finally liver reduced glutathione (Sedlak and Lindsey, 1968) levels in all the four groups of animals.

Ethical Considerations: This study was conducted according to standard procedures, international protocols and accepted principles for laboratory animal use and care conforming to ethical guidelines laid down by Institutional Animal Ethics Committee (IAEC).

Development of food products: Two food products were developed incorporating Arjuna bark and it was followed by sensory evaluation involving semi-trained panels of human subjects (Stone and Sidel, 1993).

Statistical analysis: Statistical analysis was carried out by using students't' test. Values with P<0.01 and beyond were considered statistically significant.

Results

High fat high cholesterol diet raised triglyceride slightly and cholesterol levels significantly. However, the effect was found to be reduced in animal groups fed high fat high cholesterol Arjuna Bark I and II diets in general and significantly in the latter as becomes evident from Table 2.

Table 2: Effect of different levels of Arjuna bark on high fat fed rat lipid status.

Group	Liver total cholesterol (mg / 100 of the tissue)	Serum total cholesterol (mg/100ml)	Serum triglycerides (mg/100 ml)
I	517.8±16.11	108.0±11.04	33.1±1.12
II	613.2±11.52[a]	139.4±17.80[a]	35.5±0.99[b]
III	502.5±9.05[a]	101.6±12.20[a]	31.5±1.03[b]
IV	484.5±9.25 [a]	80.57±6.41[a]	28.2±1.27[b]

Values are mean ± se of rats. group i compared with group ii, compared with group iii and iv. group i: isoenergic normal fat-cholesterol; group ii: high fat, high cholesterol; group iii: high fat, high cholesterol arjuna bark; group iv: high fat, high cholesterol arjuna bark ii; a = p<0.01: significant; b = p<0.01: non-significant

Table 3: Effect of different levels of Arjuna bark on high fat fed rat oxidative stress status

Group	Serum lipid peroxidation (nM of MDA/100ml)	Liver lipid peroxidation (nM of MDA/mg protein)	Liver GSH (mM/100g)
I	23.2±1.62	0.61±0.030	332.0±9.69
II	41.1±1.38[a]	0.87±0.031[a]	207.2±6.47[a]
III	32.6±1.22	0.66±0.028	294.7±7.52
IV	26.1±0.96[a]	0.72±0.040[a]	277.6±6.78[a]

values are mean ± se of 5 rats. group i compared with group ii, group ii compared with group iii and iv. group i: isoenergic normal fat – cholesterol; group ii: high fat, high cholesterol; group iii: high fat, high cholesterol arjuna bark i; group iv; high fat, high cholesterol arjuna bark ii a = p<0.01: significance; b = p<0.01: non-significant

Table 3 depicts the effect of feeding Arjuna bark powder at levels 5g/Kg and 10 g/Kg on the high dietary fat and cholesterol induced oxidative stress in rat blood and liver tissues. Whereas high fat diets had significantly increased the level of TBARS in both serum and liver, it

caused a decrease in liver reduced glutathione level. High fat high cholesterol Arjuna bark I and II diets tended to reverse theses trends in significant terms in almost all of these cases in references to indices associated with oxidative stress status.

Increased levels of lipids due to high dietary fat and cholesterol feeding seemed to be related to their more effective utilization in tissue lipid synthesis than that of carbohydrates, for all the alteration in the dietary fat component of high fat diet was made at the expense of carbohydrates (table 2). It is speculated that the reduction in the lipid raising effect of fat Arjuna bark I and II containing diets can be due to their capacity to increase the thermic effect of fat, thereby weaning them away from the process of tissue lipid synthesis.

In conclusion, the study seemed to reveal that high fat and high cholesterol diet and Arjuna bark intervention play the aggregative roles respectively in the context of lipid and oxidative stress status in blood and hepatic tissues, but these effects were considerably attenuated by Arjuna bark, when fed in combination with high fat high cholesterol diet. The diet containing the higher level (10g/Kg) of Arjuna bark (Arjuna bark II) proved more effective.
The second phase of this study involved developing some products in which Arjuna bark was incorporated. The purpose of developing such products using Arjuna bark was to make people partake the preventive/curative principle in the bark to the people vulnerable to chronic diseases, especially hypertension and cardiovascular diseases so as to make them take Arjuna bark over a long period as a part of their diet. In this quest two food products, namely *Arjuna Omelette* and *Arjuna En Upma* were developed using basic ingredients of *Vegetable Omelette* and *Upma* respectively to each of which 5 g of Arjuna bark was added as per the recipes given in table 4 and 5.

Table 4: Arjuna Bark – Vegetable Omlette

S. No.	Ingredients	Weight (g)
1	Bengal gram flour	45
2	Onion	15
3	Green chillies	2
4	Oil	8
5	Arjuna Bark	5
6	Tomato	25
7	Salt	To taste

Table 5: Arjuna En – Upma

S. No.	Ingredients	Weight (g)
1	Semolina	60
2	Onion	20
3	Green chillies	4.5
4	Oil	10
5	Arjuna Bark	5
6	Mustard	0.5
7	Salt	To taste

To assess the overall acceptability of the two products as evident by the data shown in table 6 and 7, indicating that Arjuna bark containing products can be substituted in place of standard recipes of *Vegetable Omelette* and *Upma*.

Table 6: Acceptability Rating of *Arjuna Omelette*

Attributes	Mean of standard recipe	Mean of test recipe
Appearance	8.8±0.83	8.9±0.89
Taste	8.8±0.81	8.6±0.60
After taste	8.8±0.01	8.2±0.67
Color	8.4±0.60	8.1±0.99
Over all acceptability	8.8±0.72	8.1±1.06

Table 7: Acceptability Rating of *Arjuna En Upma*

Attributes	Mean of standard recipe	Mean of test recipe
Appearance	8.1± 0.83	7.2±0.97
Taste	8.4±1.10	7.4±0.99
After taste	8.3±0.97	7.0± 0.83
Color	8.3±0.99	7.6±0.60
Over all acceptability	8.4±0.63	6.9±0.72

The two products were evaluated on 9 point hedonic method for the following attributes:

(i) Appearance: Appearance of the product influences the willingness to accept or reject it by the panel members. The two products *Arjuna Omelette* and *Arjuna En Upma* were liked very much and liked moderately respectively.

(ii) Taste: Taste is the most important attribute which determine the acceptability of a product. Taste wise *Arjuna Omelette* was liked very much whereas *Arjuna En Upma* was liked moderately.

(iii) After taste: After taste of a recipe is the mouth feel which one experience after eating a dish. In terms of after taste, both products were liked moderately.

(iv) Color: Color of the dish plays an important role in the acceptance of the products. Color of *Arjuna Omelette* was liked moderately while *Arjuna En Upma* liked very much.

(v) Over all acceptability: This provides the criterion about general acceptance of the products. The products showed good overall acceptability.

Thus *Arjuna Omelette* and *Arjuna En Upma* both attained good acceptability scores in the sensory evaluation phase of the study.

DISCUSSION

Arjuna bark is a widely used herbal material in traditional medicine. In the present study, its hypocholesterolemic, hypolipidemic and oxidative stress lowering has been estimated. The results concluded that Arjuna bark I and II fed diets reduced triglyceride and cholesterol moderately and significantly respectively. *T. arjuna* was found to be the most potent hypolipidemic agent and induced partial inhibition of rabbit atheroma by other markers (Shaila

et al., 1998). Shaila *et al*., (2000) also reported that the alcoholic and aqueous fractions of *T. arjuna* inhibited this rise in serum cholesterol.

Arjuna bark I and II decreased TBARS in both serum and liver significantly. Further, higher oxidative stress status of the high fat high cholesterol fed group pointed towards a possibly higher free radical activity generation resulting in peroxidative damage reflected by higher levels of the lipid per-oxidation (TBARS) and lowered one of reduced glutathione than those of normal fed groups (table 3) (Halliwell, 1996; Benzie, 1996; Kasugi *et al*., 1993; Beutler, 1989). However, feeding of the two levels of Arjuna bark (I and II) along with high cholesterol diet to the two groups (C or D) effectively diminished the oxidative stress build up recovering all the indices close to those of normal fat fed control group. Das *et al*., (2010) also reported that dehydration induced oxidative stress and uremia in male rats may be protected by using the arjuna plants extract. According to Raghavan and Kumari (2006), ethanolic extract of the same plant exhibited the antioxidant activity through correction of oxidative stress. Raj *et al*., (2012) also concluded that hydroalcoholic extract of *Terminalia arjuna* bark at the dose levels of 250 and 500 mg/kg showed significant antioxidant potential in isolated perfused rat kidneys.

Verma and Vinayak (2009) reported the antioxidant action of aqueous extract of *T. arjuna*, which may play a role in the anti carcinogenic activity by reducing the oxidative stress along with inhibition of anaerobic metabolism. Manna *et al*., (2006) demonstrated that the aqueous extract of the bark of TA could protect the liver and kidney tissues against CCl_4-induced oxidative stress probably by increasing antioxidative defense activities. Khan *et al*., (1997) also concluded the same where diets encompassing natural antioxidants have been reported to reclaim the normal pro and antioxidant steady state of the animals. Likewise, studies of Sharma and Sharma (2001) also suggest that apart from reducing lipid levels, dietary antioxidants also support endogenous antioxidants in their oxidative stress reducing endeavors.

Both developed products *Arjuna Omelette* and *Arjuna En Upma* were well acceptable but former one was more liked than latter one on the basis of overall acceptability. A study was conducted by Kulkarni (2003) to explore the utility of underutilized leafy vegetables to enrich routine diets for nutrition security. Five underutilized leafy vegetables were selected on the basis of the micronutrient profile (drumstick, *chakramuni*, bengal gram leaves, *chandanabatta* leaves and *sambar soppu*) and 14 value added traditional foods were developed by incorporating these at different levels (10 or 25 or 50%). The products were evaluated for sensory characteristics using nine point hedonic method by 10 semi trained judges. The results showed that coconut *chutney* with *sambar soppu* scored highest for all sensory attributes followed by *bisebilebath* and little millet *upma* with drumstick leaves and the least scores were obtained for barnyard millet *upma* with drumstick leaves.

CONCLUSIONS

To sum up, the following inference can be drawn from the two phases of the study. The phase I result provided towards arjuna bark being a hypocholesterolemic and hypolipidemic agent. Further, it also appeared to be helpful in mitigating oxidative stress induced by high fat high cholesterol diet. The two recipes developed with incorporation of Arjuna bark elicited good acceptability response in sensory appraisal studies and as such have the potential to deliver in terms of lipid and oxidative stress lowering effects in food based interventions to the vulnerable population.

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