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Role of Arjuna bark containing polyherbal formulation on oxidative stress parameters in hyperlipidemic chick model

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Abstract
Oxidative stress plays a major role in initiation and progression of hyperlipidaemia-induced atherosclerosis. Standard pharmacotherapy of dyslipidemia includes treatment with HMG Co-A reductase inhibitors, anion exchange resins, fibrates, nicotinic acid and its derivatives. While, the pathophysiological processes involved in hyperlipidemia leading to atherosclerosis have to be modulated, since, none of these drugs (except statins) till date has other pharmacological effects than causing hypolipidemia. Many plants are of global attention, with significant antioxidant, anti inflammatory and antilipidemic activity. Surprisingly their combined formulation and its effect in lipid lowering and inflammation have not been investigated. A study was carried out to investigate the influence of polyherbal formulation on certain oxidative stress parameters in hyperlipidemic chick model, which will help in further exploration of similar effects in human conditions.

Keywords: oxidative stress, atherosclerosis, dyslipidemia, hyperlipidemia

Introduction
Atherosclerosis is a chronic disease with complex etiology and considered as a primary cause of CAD. Hyperlipidaemia along with concurrent pathophysiological processes such as, endothelial dysfunction, inflammation, oxidative stress, is a harbinger of atherosclerosis. Oxidative stress plays a major role in initiation and progression of hyperlipidaemia-induced atherosclerosis. This is evidenced by the fact that oxidative modification of LDL-C is an important feature in the development of atherosclerotic plaque.
In the light of lack of information on the influence of aqueous extract of *Terminalia arjuna* bark, *Inula racemosa* root, *Tribulus terrestris* seeds, *Rhododendron arboretum* flower, *Garcinia indica* fruit juices on oxidative stress parameters in chick model, this study was undertaken to investigate the influence of polyherbal formulation on certain oxidative stress parameters in hyperlipidemic chick model, which will help in further exploration of similar effects in human conditions.

Materials and methodology
Formulation of Cardorium plus (test drug Composition for 10ml decoction).

Plant	part	quantity
Terminalia arjuna	Bark	250mg
Inula racemosa	root	150mg
Tribulus terrestris	seeds	250mg
Rhododendron arboretum	flower	150mg
Garcinia indica	Fruit	200mg
Pure water		Q.S.
Preservative		Q.S

Methods
Animals
Thirty two broiler chicks weighing (100- 150 gm) were procured, randomly divided into 4 groups of 8 each, marked to permit individual identification and kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

For initial three weeks, round the clock a temperature of 40±2ºC was maintained using 100 candles bulbs. The animals were kept on standard diet and water *ad libitum.*

Following groups were considered for the study
Control: normal diet and water (control) for 8 weeks.

Group cholesterol: 2% cholesterol enriched diet for 8 weeks.

Group L: 2% cholesterol enriched diet + lovastatin – (4.65mg/kg)

Group S+R: 2% cholesterol enriched diet + Test drug (Cardorium plus - 0.775 ml/kg)

Group A: 2% cholesterol enriched diet +Standard drug (Ascorbic acid-19.37mg/kg)

Chick's diet: (per 100gms)
Corn - 63.81, Soybean meal - 30.13, Wheat bran - 0.75, Dicalcium phosphate - 1.03, Limestone - 1.24, Vit. Min. Premix - 0.50, Salt - 0.31, Veg. oil - 2.20, DL- Methionine - 0.03.
High fat diet was prepared by mixing 100 Gms of normal diet with 2 Gms of cholesterol (2%)

Dose and route of administration
The cardorium plus dose for chick = 10x0.031=0.31 for 400 gms chicken, 0.775 ml is the dose for 1kg chick.
The lovastatin dose for chick = 60x0.031=1.86 for 400 gms chicken, 4.65mg is the dose for 1kg chick. For the preparation of suspension 0.5% carboxy methyl cellulose was used.
The ascorbic acid dose for chick = 250x0.031=7.75for 400 gms chicken, 19.37 mg is the dose for 1kg chick.
Drugs are administered through oral route. At the end of 8 weeks study blood was collected by using 2 ml syringe blood from the femoral vein. Serum was separated from blood and various biochemical parameters were measured.

Biochemical parameters
Estimation of TBARS (Ohkawa *et al*., 1979)
Procedure
To 0.2 ml of plasma sample, 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid (pH 3.5) were added. Volume was made upto 4 ml with double distilled water and heated at 95^0 C for 60 min. After cooling, 1 ml of double distilled water and 5 ml of butanol: pyridine mixture was added. The solution was shaken vigorously in a vortex and centrifuged at 1000 x g at room temperature for 10 min at room temperature. Organic layer was separated and absorbance was read at 532 nm in the spectrophotometer (UV-VIS Spectrophotometer, SL 150, Elico, Hyderabad).

Estimation of Erythrocyte GSH (eGSH) level (Ellman, 1959)
Procedure
To 2ml of buffer was added 100µl of sample followed by the addition of 500µl DTNB and volume is made up to 3ml with distilled water. Mixed well, kept for incubation for 10 minutes and absorbance was read at 412nm against the reagent blank.

Estimation of Erythrocyte Catalase (eCAT) activity (Aebi, 1974)
Procedure
To 0.05 ml of sample was added 2ml of phosphate buffer, 1 ml H_2O_2 was added and extinction was read at 240 nm at 15 sec. interval for a total of 30 sec.

Estimation of erythrocyte SOD (eSOD) activity (Stefan marklund and gudrun marklund., 1974)
Procedure
To 0.9 ml buffer was added 0.05 ml of 4 mM pyrogallol followed by addition of 0.05 ml of sample. Zero reading was taken by adding 0.05 ml of 4 mM pyrogallol to 0.95 ml buffer. Absorbance was read at 420nm for 3min at 30 sec intervals.

Estimation of hemoglobin (Crest biosystems, coral groups)
Procedure
20µl of sample/ standard was added to 5ml of Drabkin's solution and mixed well. After an incubating at room temperature for 5 minutes, absorbance was read against distilled water.

Wave length: 540nm

Cuvette: 1cm light path

Temperature: 20-25ºC

Measurement: against reagent blank

Results

Table 1: Control group animals treated with normal diet (Group I)

S. No	Weight of the animal (kg)	Erythrocyte TBARS Concentration (nmol/ml)	Erythrocyte Catalase concentration (k/g hb)	Erythrocyte SOD Concentration (µg/hb)	Erythrocyte GSH Concentration (umol/g hb)
1	2.9	1.46	4328	3415.4	8.31
2	3.1	1.62	4561.2	3326.3	11.62
3	3.3	1.22	4456.4	3612.4	9.43
4	3.2	1.42	4139.3	3516.2	10.86
5	2.7	1.34	4936.6	3745.8	8.31
6	3.0	1.52	4561.2	3485.4	12.38
7	2.6	1.42	4716.4	3516.2	11.62
8	3.1	1.42	4139.3	3176.8	9.43
Mean±S.D	2.9±0.2	1.42±0.1	4355±165	3474.3±50.6	10.24±1.58

Table 2: Fat group animals treated with cholesterol diet (Group II*)*

S. No	Weight of the animal (kg)	Erythrocyte TBARS Concentration (nmol/ml)	Erythrocyte Catalase concentration (k/g hb)	Erythrocyte SOD Concentration (ug/hb)	Erythrocyte GSH Concentration (umol/g hb)
1	3.9	2.81	2915.1	2368.4	6.46
2	3.8	2.94	2365.2	2572.6	6.12
3	3.4	2.72	2516.3	2251.3	6.46
4	4.2	3.21	2915.1	2759.2	5.89
5	3.6	3.34	2365.2	2635.9	7.43
6	3.9	2.86	2614.1	2492.1	6.79
7	3.6	3.16	2841.3	2572.6	6.12
8	4.4	2.92	2516.3	2251.3	5.32
Mean±SD	3.8±0.3	2.99±0.2	2631±231	2487.9±183	6.32±0.62

Table 3: Animals treated with cholesterol diet & cardorium plus (Group III)

S. No	Weight of the animal (kg)	Erythrocyte TBARS Concentration (nmol/ml)	Erythrocyte Catalase concentation (k/g hb)	Erythrocyte SOD Concentration (ug/hb)	Erythrocyte GSH Concentration (umol/g hb)
1	3.9	2.14	3816.2	3253.2	8.31
2	3.8	2.09	3429.3	3321.4	8.96
3	3.5	1.92	3616.5	3764.7	9.43
4	4.2	1.88	3429.3	3486.2	8.01
5	3.9	1.88	3551.3	3852.6	9.62
6	3.6	2.28	3816.2	3764.7	8.56
7	3.5	2.06	3721.7	3253.2	8.96
8	3.8	1.96	3429.3	3321.4	10.41
Mean±S.D	3.7±0.2	2.02±0.14	3601±168	3502.1±253	9.032±0.77

Table 4: Animals trated with cholesterol diet & lovastatin (Group IV)

S. No	Weight of the animal (kg)	Erythrocyte TBARS Concentration (nmol/ml)	Erythrocyte Catalase concentation (k/g hb)	Erythrocyte SOD Concentration (ug/hb)	Erythrocyte GSH Concentration (umol/g hb)
1	3.6	2.46	3126.4	2759.2	8.01
2	3.2	2.52	2971.7	2651.2	8.31
3	2.9	2.56	2841.3	2863.8	7.24
4	3.3	2.49	3126.4	3053.9	7.68
5	3.5	2.56	3049.4	3253.9	7.89
6	3.6	2.41	2841.3	2863.8	8.31
7	4.1	2.46	3126.7	3486.2	7.68
8	3.9	2.52	2971.7	3164.7	7.42
Mean±S.D	3.5±0.3	2.49±0.05	3006.8±120	3012±279	7.81±0.38

Table 5: Animals treated with cholesterol diet & ascorbic acid (Group V)

S. No	Weight of the animal (kg)	Erythrocyte TBARS Concentration (nmol/ml)	Erythrocyte Catalase concentation (k/g hb)	Erythrocyte SOD Concentration (ug/hb)	Erythrocyte GSH Concentration (umol/g hb)
1	3.9	1.67	4139.3	4126.8	9.43
2	3.8	1.61	3816.2	3852.9	10.86
3	3.7	1.76	3616.5	3321.4	9.81
4	4.2	1.72	4328.1	3253.2	10.41
5	3.6	1.78	4139.3	4126.8	11.62
6	3.4	1.67	3712.7	3671.8	9.81
7	4.6	1.61	3816.2	3486.2	10.32
8	4.9	1.72	3616.5	3321.4	11.64
Mean±S.D	4.0±0.5	1.69±0.06	3898.1±269	3645±357	10.4±0.8

Conclusion

The present study proved that Cardorium plus has preventive role in hyperlipidemia associated changes in oxidative stress. The cardorium plus antioxidant activity was comparable with that of standard drug, ascorbic acid. However, the elucidation of the exact mechanism/s of Cardorium plus effects on hyperlipidemia induced oxidative stress changes needs further studies.

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