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ANTIDYSLIPIDEMIC AND ANTIOXIDANT ACTIVITIES OF DIFFERENT FRACTIONS OF *TERMINALIA ARJUNA* STEM BARK

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ABSTRACT

Terminalia arjuna (*T. arjuna*) stem bark was successively extracted with petroleum ether (A), solvent ether (B), ethanol (C) and water (D). The lipid lowering activity of these four fractions A, B, C, and D was evaluated *in vivo* in two models viz., triton WR-1339 induced hyperlipemia in rats as well as fructose rich high fat diet (HFD) fed diabetic- dyslipidemic hamsters. Hyperlipidemia induced by triton caused marked increase in the plasma levels of total cholesterol (Tc), triglyceride (Tg) and phospholipids (PL) in rats. After treatment with *T. arjuna* fractions A, B, C, and D at the doses of 250 mg/kg per oral (p.o.), only the ethanolic fraction (C) exerted significant lipid lowering effect as assessed by reversal of plasma levels of Tc, Tg and PL in hyperlipidemic rats. In another experiment, feeding with HFD produced marked dyslipidemia as observed by increased levels of plasma Tc, Tg, glucose (Glu), glycerol (Gly) and free fatty acids (FFA) in hamsters. After treatment with *T. arjuna* fractions at the doses of 250 mg/kg p.o. only two fractions (B and C) could exert significant lowering in the plasma levels of lipids and Glu. in dyslipidemic hamsters. *In vitro* experiment *T. arjuna* fractions at tested concentrations (50-500 µg/ml) inhibited the oxidative degradation of lipids in human low density lipoprotein and rat liver microsomes induced by metal ions. These fractions when tested against generation of oxygen free radicals at the concentrations (50-500 ig/ml), counteracted the formation of superoxide anions (O^{-2}) and hydrodyl radicals (OH) in non enzymic test systems. The efficacy of *T. arjuna* fractions as antidyslipidemic and antioxidant agents was found, fraction C> fraction B> fraction A.

KEY WORDS

Antidyslipidemic activity, antioxidant activity, *Terminalia arjuna* oxygen free radical, triton model, hamster model.

INTRODUCTION

The bark of *Terminalia arjuna* (family Combretaceae) is one such Ayurvedic remedy that has been mentioned in many ancient Indian medicinal literature including Charaka Samhita and Astang Hridayam, to possess cardio protective property (1). It is an essential ingredient of many potent Ayurvedic preparations sold as cardiotonics (2). The plant is found in plenty throughout Indo sub Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi, Deccan region mainly along riverside, riverlets and ponds. A number of experimental and clinical studies have proved that dried bark powder of this plant have potent hypolipidemic and cardioprotective activity (3-7). The bark powder of *T. arjuna* has also been found to improve antioxidant status in the patients of coronary heart disease and these beneficial effects may be related to its high flavonide content (8). It has been well documented that bark extract contains acids (arjunic acid, terminic acid), glycosides (argentine arjunosides I-IV), strong antioxidants (flavones, tannins, oligomeric proanthocyanidins), minerals etc, but not much is known about the specific biological activity of individual constituents of this plant. Few of the active compounds from *T. arjuna* bark have been isolated and shown to possess antimutagenic and anticarcinoginic activity (9, 10). Arjunolic acid, a new triterpene isolated from the bark of *T. arjuna* have been reported to have antioxidant and cardioprotective activity (11). But the fraction type

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of *T. arjuna* bark extract having the high degree of antioxidant and antidyslipidemic activity is not well known. Therefore the present study has been undertaken to evaluate the antidyslipidemic activity of different solvent fractions of bark extract of *T. arjuna* bark powder in triton and high fat diet (HFD) fed hamster model. The antioxidant activity of these fractions was also evaluated by generating free radicals *in vitro* in the absence and presence of *T. arjuna* fractions.

MATERIALS AND METHODS

Preparation of fractions of *T. arjuna*

Fresh *T. arjuna* stem bark was collected, identified pharmacognostically in the Botany division of the Institute. Bark powder of *T. arjuna* was successively extracted with petroleum ether (fraction-A), solvent ether (fraction-B), alcohol (fraction-C) and aqueous (fraction-D) as described by Row *et al.* (12). These fractions were used for *in vivo* and *in vitro* studies.

Animals

Rats (Charles Foster strain, male, adult, body wt 200-225 g) and hamster (Golden Syrian, male, adult, body wt. 120-130 g) were kept in a room with controlled temperature at 25-26°C, humidity 60-80% and 12/12 hours light/dark cycle (light on from 8.00 AM to 8 PM) under hygienic conditions. Animals were acclimatised for one week before starting the experiment. The animals had free access to the normal diet and water.

Lipid lowering activity in Triton induced hyperlipidemic rat model

Rats were divided into six groups- control, triton induced, triton plus *T. arjuna* fractions A, B, C and D treated groups containing six animals in each group. In the acute experiment of 18 hours, hyperlipidemia was developed by administration of triton WR-1339 (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 400 mg/kg, b.w. intraperitoneally to animals of all the groups except the control. *T. arjuna* fractions were macerated with gum acacia, suspended in water and fed simultaneously with triton at a dose of 250 mg/kg p.o. to the animals of *T. arjuna* fractions treated groups and diet was withdrawn.. Animals of control and triton group without treatment with *T. arjuna* fractions were given same amount of gum acacia suspension (vehicle). After 18 hours of treatment the animals were anaesthetised with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and 1ml blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated eppendorf tubes (3.0 mg/ml blood). The blood was centrifuged at 2500 x g for 10 minutes at 4°C and plasma was separated. Plasma was diluted with normal saline in a ratio of 1:3 and used for the analysis of total cholesterol (T-chol), triglyceride (Tg) and phospholipid (PL) by standard enzymatic methods (13, 14, 15) using auto-analyser (Beckmann Coulter model "Synchron- CX-5 Clinical System" USA). Kits were purchased from Beckman Coulter International, USA.

Antidyslipidemic activity in high fat diet fed hamster model

In Hamsters, dyslipidemia was developed by feeding them with high fat diet (HFD) purchased from Research Diet Inc,New Brunswick USA (product code No. D-99122211) containing fructose (9% w/w), deoxycholic acid (0.45% w/w), cholesterol (0.45% w/w), powdered hamster chow diet (64% w/w) and coconut oil (26% w/w). Hamsters were divided into six groups - control, HFD fed, HFD fed and *T. arjuna* fraction-A, B, C and D treated groups containing eight animals in each. HFD (9.0g / animal) was given daily from day1 to day 10 to animals of all the groups except control. Animals had free access to the diet and the daily diet consumed by animals was calculated by subtracting the left over diet on next day from the added diet on previous day. Control group received the normal hamster chow. *T. arjuna* fractions were given orally at a dose of 250 mg/kg b.w. once a day from day 4 to day 10 to all groups except the animals of control and HFD fed group, to them same amount of vehicle (as mention above) was given. Body weight of animals was recorded daily. On tenth day, two hours after the dosing, animals were anaesthetised and blood was collected in EDTA coated eppendorf tubes as well as in an another similar tube (EDTA coated) having 120 ml of NaF solution (45 mg/ml). The blood was centrifuged at 2500xg rpm for 10 minutes at 4°C and plasma was separated. Plasma without NaF solution was used for the analysis of T-chol (13), Tg (14) and HDL-cholesterol (HDL-chol) (16) and the plasma with NaF solution was used for the analysis of glucose (Glu), glycerol (Gly) and fatty acids (FFA) by standard enzymatic methods (16,17,18,19) using auto-analyser. All assay kits except of FFA were purchased from Beckman Coulter International, USA and assay Kit for FFA was purchased from Wako Pure Chemical Industries Ltd., Osaka Japan.

Antioxidant activity

LDL oxidation : Serum was separated from the blood of normolipemic donors who were fasted overnight and fractionated into very low-density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by ultracentrifugation (20). The lipoproteins preparations were dialysed

against 150 mM NaCl containing EDTA (0.02 % w/v) in presence of N_2 gas in cold. The purity of LDL was checked on polyacrylamide gel electrophoresis. LDL (0.71 mg) and $CuCl_2.2H_2O$ (10 µM) in the absence or presence of *T. arjuna* fractions A, B, C and D (50-500 µg/ml) in 50 mM phosphate buffer saline (pH 7.4) to a final volume of 1.5 ml, was incubated at 37°C for 16 hours. The level of lipid peroxides in unoxidised LDL, oxidised LDL with Cu^{++} in the absence or presence of *T. arjuna* fractions was assayed (21) as thiobarbuteric acid reactive substances (TBARS). Briefly the reaction mixture contained 0.5ml SDS (8% w/v), 0.5 ml glacial acetic acid, 1.5 ml TBA (0.8% w/v) was heated in a boiling water bath for one hour. After cooling upto room temperature optical density of reaction mixture was read at 532 nm with respective reagent blank. The level of lipid peroxide as n mol of malondialdehyde formed was calculated by taking absorption Coefficient of MDA as 1.78 $x10^5$ cm^{-1} M^{-1} mg protein.

Lipid peroxidation in Microsomes : Lipid peroxidation in microsomes was induced by non-enzymic reactants (22). Briefly rat liver microsomes (protein 2.0 mg), $FeSO_4.7H_2O$ (2 µM) and sodium ascorbate (10 µM) in absence or presence of *T. arjuna* fractions (50-500 mg/ml) in 100 mM phosphate buffer, pH 7.8 to a final volume of 2 ml were incubated at 37°C for 90 minutes. Reference tubes and reagent blank were also run simultaneously. After incubation, all the sets were assayed for lipid peroxide content by their reaction with thiobarbituric acid as mentioned above (21). The lipid peroxide content in microsomes was expressed as n mol. MDA formed/mg protein.

Generation of oxygen free radicals - Superoxide anions (O^{-2}) were generated enzymatically (23) by xanthine (160 mM), Xanthine oxidase (0.04 units) and Nitroblue tetrazolium (320 µM) in absence or presence of *T. arjuna* bark fractions (50-500 µg/ml) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixture was incubated at 37°C. After 30 minutes the reaction was stopped by adding 0.5 ml glacial acetic acid and the amount of formazone formed was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as $7.2x10^3$ M^{-1} cm^{-1}. In another set of experiment, effect of *T. arjuna* fractions on generation of hydroxyl radicals (OH-) was also studied by non-ezymic reactants (24). Briefly OH^- were generated in a non-enzymic system comprised of deoxyribose (2.8 mM), $FeSO_4.7H_2O$ (2 mM), Sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of *T. arjuna* bark fractions (50-500 mg/ml) were incubated at 37°C for 90 minutes. Reference tubes and reagent blanks were also run simultaneously. Malondialdehyde (MDA) content in both experimental and reference tubes were estimated spectrophotometrically by thiobarbuteric acid method as mentioned above (21).

Statistical evaluation

Data were analysed using student's t-test. The hyperlipidemic groups were compared with control and *T. arujna* fractions treated groups. Similarly the generation of oxygen free radicals and lipid peroxidation in LDL and microsomes with different fractions of *T. arjuna* were compared with that of their formation without fractions. $P<0.05$ was considered to be significant.

RESULTS

Effect of *T arjuna* fractions on hyperlipidemia

Administration of triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the plasma levels of Tc (5.90 fold), Tg (4.59 fold) and PL (7.89 fold) as compared to control (Table 1). Treatment of hyperlipidemic rats with *T. arjuna* fractions A, B, C and D at the dose of 250 mg/Kg p.o. reversed the plasma levels of lipid but with varying extents. The effect of ethanolic fraction (fraction C), causing a decrease in plasma levels of Tc, Tg and PL by 31, 40 and 18% respectively as compared to triton was more significant than other fractions. The order of lipid lowering activity by these fractions in above model was fraction C> fraction D> fraction B> fraction A (Table 1). In another experiment dyslipidemia in hamster was produced by feeding them with fructose rich HFD. The plasma levels of Tg, Tc, HDL-chol, glucose, glycerol and FFA of these animals were found to be elevated by 106, 300, 30, 54, 30 and 87% respectively as compared to control. Administration of *T. arjuna* fractions at the dose of 250 mg/Kg p.o. simultaneously with HFD in hamsters lead to lowering in the plasma levels of lipid and the effects of fraction B (solvent ether) and that of fraction C (ethanol) were more pronounced as these animals were observed with decrease in levels of Tg (28 and 28%), Tc (37-48%) and glycerol (34 and 39%) respectively as compared to dyslipidemic group Table 2). HFD caused increase in the plasma level of HDL-chol. which was further increased (20%) after treatment with *T. arjuna* fraction C (ethanol) but other fractions failed to shown any significant increase in above parameter in dyslipidemic hamster. Furthermore HFD caused increase in the level of plasma glucose by 54% as compared to

Table 1. Effect of *T. arjuna* bark fractions on biochemical parameters in hyperlipidemic rats

Treatments	Total Cholesterol	Triglycerides	Phospholipids
Control	85.50± 6.35	82.15± 7.76	70.60± 5.20
Triton treated	504.86± 33.95***	373.71± 36.58***	557.06± 50.85***
Triton + Fraction A treated	484.42± 18.37^{NS}	264.90± 25.92**	480.00± 38.43*
Triton + Fraction B treated	423.80± 27.25*	328.33± 29.40^{NS}	550.72± 37.69^{NS}
Triton + Fraction C treated	348.48± 30.84***	224.89± 11.78***	454.70± 33.55**
Triton + Fraction D treated	399.67± 28.83*	299.90± 27.21^{NS}	504.22± 50.12^{NS}

Fraction-A (petroleum ether), Fraction-B (solvent ether) and Fraction-C (ethanol) Fraction - D (Aqueous)

Unit: mg/dl. Each value is mean ± SD of six rats. P*< 0.05, **< 0.01, ***< 0.001, NS = non significant. Hyperlipidemic group was compared with Control, hyperlipidemic + *T. arjuna* treated with hyperlipidemic.

control and these levels were observed with a slight decrease (15-16%) under the action of *T. arjuna* fraction A, C and D. Treatment with *T. arjuna* did not effect the body weight, food intake and plasma level of FFA in dyslipidemic animals.

Effect of *T. arjuna* fractions on lipid peroxidation and oxygen free radical generation *in vitro*

Lipid peroxide content in unoxidized LDL was 0.70 ± 0.04 n mol MDA/mg protein. Oxidation of LDL in the absence of metal ions caused formation of lipid peroxides (1.02 ± 0.50 n mol MDA/mg protein ; data not shown). However incubation of LDL with Cu^{+2} caused a mark increase in lipid peroxide by 20 to 23 folds (Table 3). Addition of T. arjuna fractions (50-500 µg/ml) inhibited the Cu^{++} mediated lipid peroxidation of LDL in concentration dependent manner. At peak levels of 500 µg/ml, the fraction A, B, C or D inhibited LDL peroxidation by 22, 76 79 and 31% respectively. It was also observed that normal rat liver microsomes containing 3.17 ± 0.35 n mol MDA/mg protein, when challenged with the reactions (Fe^{+2}-sodium ascorbate) employed in non-enzymic system for peroxidation, increased the levels of lipid peroxides by 2.0-2.5 folds (Table 3). Addition of *T. arjuna* fractions (50-500 µg/ml) in above system made microsomes less susceptible against oxidative degradation of their lipids and at peak concentration of 500 µg/ml fraction A, B, C or D could protect peroxidation in microsomes by 20, 45, 50 and 13% respectively (Table 3). Furthermore, the scavenging potential of *T. arjuna* fractions at 50-500 ìg/ml against formation of O_2^- and OH^- in non enzymic systems were also studied (Table 3). The significant decrease in superoxide anions (27 and 70%) by fraction A (petroleum ether) and fraction C (ethanol) and hydroxyl radicals (17, 41, 40 and 19%) by fractions A,B,C or D was found at concentrations of 500 µg/ml. Solvent ether and ethanolic fraction (fraction B and C) of *T. arjuna* showed more antioxidant activity in above test systems as compared to that of fractions A and D.

DISCUSSION

The involvement of hydroxyl free radicals (OH^-) is a major causative factor for peroxidative damage to lipoproteins, which is responsible for initiation and progression of atherosclerosis in hyperlipidemic subjects (25). Hyperlipidemia may also induce abnormalities with oxidation of fatty acids leading to the formation of ketone bodies as well as making liver and muscle resistance to insulin which initiate and progress diabetes in patients (26). Furthermore, due to hyperglycaemia, increase in non enzymic glycosylation occurs, accompanied with glucose oxidation and these reactions are catalysed by Cu^{+2} and Fe^{+2}, resulting in formation of O_2^- and OH^- radicals which further accelerates the risk of cardiac diseases in dyslipidemia (27).

To overcome these ailments, a drug having multifold properties such as lipid lowering, antidiabetic and antioxidant activities together is in great demand. *T. arjuna* bark have been reported earlier to possess both the lipid lowering and antioxidant activities. In the present study, we have investigated these properties in different solvent fractions of this plant material and attempted to isolate active fraction (s) and effective molecules. Both solvent ether and ethanolic fractions (fraction B and C) caused significant decrease in the plasma levels of lipids in triton as well as in HFD fed models of hyperlipidemia. Triton WR-1339 acts as surfactant, suppresses the action of lipases and block the uptake of lipoprotiens from circulation by

Table 2. Effect of *T. arjuna* bark fractions on physical and biochemical parameters in dyslipidemic hamsters

Treatment	Body Weight[a]	Food intake[a]	Tri-Glyceride[b]	Total Cholesterol[b]	HDL-Cholesterol[b]	Glucose[b]	Glycerol[b]	Free fatty acids[c]
Control	148.50 ± 9.60	10.50 ± 4.90	177.66 ± 24.11	89.33 ± 9.33	44.22 ± 12.79	66.00 ± 15.13	63.40 ± 16.71	1.83 ± 0.17
Dyslipdemic	159.60 ± 10.00^{NS}	8.33 ± 1.00^{NS}	366.75 ± 63.40***	300.00 ± 65.00**	57.058 ± 2.30**	102.00 ± 25.90**	158.40 ± 15.00**	3.43 ± 0.04***
Fraction A	160.80 ± 5.20^{NS}	8.67 ± 2.30^{NS}	263.60 ± 0.70**	352.30 ± 93.80^{NS}	58.16 ± 4.50^{NS}	86.60 ± 22.10^{NS}	112.00 ± 16.00***	3.46 ± 0.08^{NS}
Fraction B	158.60 ± 7.00^{NS}	9.82 ± 0.68^{NS}	262.40 ± 56.40*	189.00 ± 51.90^{NS}	62.00 ± 10.50^{NS}	105.80 ± 17.00^{NS}	104.00 ± 25.00***	3.50 ± 0.11^{NS}
Fraction C	157.00 ± 8.22^{NS}	10.33 ± 2.13^{NS}	264.00 ± 65.50^{NS}	157.20 ± 38.00^{NS}	68.50 ± 6.83***	87.16 ± 23.00^{NS}	97.00 ± 15.00***	3.54 ± 0.74^{NS}
Fraction D	150.50 ± 7.96^{NS}	8.30 ± 0.00^{NS}	281.66 ± 70.30*	301.33 ± 69.04^{NS}	55.16 ± 6.21^{NS}	85.83 ± 11.37*	105.00 ± 15.87***	3.11 ± 0.25^{NS}

Fraction-A (petroleum ether), Fraction-B (solvent ether), Fraction-C (ethanol) and Fraction - D (Aqueous)

Units: a, g; b, mg/dl; c, mM. Each value is the mean ± SD of 8 hamsters. P*< 0.05, **< 0.01, ***< 0.001, NS = non significant. Dyslipidemic group was compared with Control, dyslipidemic + *T. arjuna* treated with dyslipidemic.

extrahepatic tissues resulting in an increase in the levels of circulatory lipid (28). These test samples inhibited cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton-induced hyperlipidemia. We have successfully used this model for evaluation of lipid lowering activity of *T.arjuna* bark and other natural products (4). We have employed HFD induced diabetes-dyslipidemia in hamster, which is a very suitable model for screening of drugs for the regulation of lipid and carbohydrate matabolism (29, 30). Feeding with solvent ether and ethanolic fractions of *T.arjuna* exhibited lowering of plasma lipids and glucose, suggesting that this property of test samples is mediated through peroxisomes proliferater activated receptor-α subtype (PPAR-α) in diversified manner by catabolizing Tg, upregulating lipoprotein lipases to rid off dietary load, and improve the HDL levels for the maintenance of lipid- glucose homeostasis in hamster model (31, 32).

To assess the antioxidant activity of *T. arjuna* fractions, we have used Cu^{+2}/ Fe^{+2} induced LDL and rat liver microsomes for oxidation as *in vitro* model of nonenzymic stimulation of lipid peroxidation mediated by the action of OH^{-} radicals (33, 34). In the present study solvent ether and ethanolic fractions of *T. arjuna* inhibited the peroxidative degradation of LDL and microsomal lipids as well as suppressed the generation of OH^{-} radicals in above tests. These properties of the fractions as antioxidant and scavenger of oxygen free radicals appears to be mediated through activity like metal ion chaelators and xanthine oxidase inhibitors (34).

We have followed the extraction procedure as described by Row *et al.* (12) who have reported that petroleum ether and solvent ether fractions of *T. arjuna* mainly contain plant sterols (β-sitosterol) and arjunic acid, respectively whereas ethanotic fraction was enriched with derivatives of arjunic acid like arjunoglycoside (I, II, II and IV), arjungenin, arjunolone, arjunetin, tanins, ellagic acid and other (35). Some pure compounds namely Arjunolic acid (11), Terminoside (36), Ellagic acid (37,38) and Tanins (9) have been found to possess cardioprotective, antiplatelet aggregative, nitric oxide suppressant, antioxidant, membrane stabilizing, hepatoprotective and anti-cancer activities.

Our data showed that both ethanol and solvent ether fractions of *T. arjuna* exerted lipid lowering activity *in vivo*. This suggests that arjunic acid as well as its derivatives when undergo

Table 3. Antioxidant activity *T. arjuna* fractions *in vitro*

Treatment	Concentration (µg/ml)	LDL-Oxidation[a]	Microsomal Lipid per oxidation[a]	Formation of super oxide anions[b]	Formation of Hydroxyl Radicals[c]
T.arjuna fraction-A	None	20.44±3.14	6.42±0.54	15.01±1.96	43.61±1.54
	50	18.20±2.60^{NS}	6.04±0.12^{NS}	12.61±2.35^{NS}	42.21±1.95^{NS}
	125	16.83±3.00^{NS}	5.88±0.11^{NS}	12.15±3.00^{NS}	40.02±1.29^{NS}
	250	16.55±2.66^{NS}	5.40±0.21^{NS}	11.68±1.60^{NS}	38.25±1.94^{NS}
	500	16.00±3.25^{NS}	5.15±0.13*	11.03±2.00*	36.00±10.25^{NS}
T.arjuna fraction-B	None	21.68±3.53	6.73±0.68	15.29±2.24	41.56±3.42
	50	18.20±3.30^{NS}	5.86±0.12*	14.47±2.35^{NS}	31.51±0.97*
	125	13.60±2.50*	5.48±0.30*	14.12±3.00^{NS}	27.25±1.29**
	250	9.20±2.25*	4.17±0.35***	14.03±1.90^{NS}	25.67±0.28**
	500	5.16±1.00***	3.72±0.17***	14.01±2.25^{NS}	24.61±0.65***
T.arjuna fraction-C	None	22.70±4.36	7.87±1.31	14.47±2.98	42.52±1.73
	50	17.25±4.00^{NS}	5.84±0.44*	8.24±1.00*	38.51±0.43^{NS}
	125	11.00±2.20**	4.98±0.42**	6.71±1.80*	31.77±0.37*
	250	8.50±1.30**	4.35±0.21**	6.23±2.00*	28.99±0.71*
	500	4.75±0.85***	3.92±0.24**	4.28±0.85**	25.50±0.25***
T.arjuna fraction-D	None	23.67±4.87	6.40±0.98	14.03±2.43	40.49±3.39
	50	21.00±4.00^{NS}	$^{6.29}$±0.26^{NS}	13.33±3.25^{NS}	33.95±2.19*
	125	19.25±3.00^{NS}	6.04±0.21^{NS}	12.96±2.55^{NS}	33.33±1.27*
	250	18.00±2.60^{NS}	5.80±0314^{NS}	12.26±3.15^{NS}	33.30±0.80*
	500	16.30±3.50^{NS}	5.57±0.18^{NS}	11.70±3.45^{NS}	32.69±0.41*

Fraction-A (petroleum ether), Fraction-B (solvent ether) and Fraction-C (ethanol) Fraction-D (Aqueous) Units: a, nmol MDA /mg protein; b, nmol formazon formed / minute; c, n mole MDA/hr. Each value is the mean ± SD of 4 separate observations.

P*< 0.05, **< 0.01, ***< 0.001, NS = non significant as compared to the systems without drug treatment.

biotransformation through hepatic drug metabolising cascade, produce common active molecules which may be responsible for lipid lowering activity *in vivo*. The quantity of arjunic acid in solvent ether fraction was comparatively very less than those of its derivative in ethanolic fraction and due to this, at the same doses ethanolic fraction was more effective than solvent ether fraction. More work on drug metabolism and to assess the biological activity *in vivo* and *in vitro* of *T. arjuna* fractions is under progress to substantiate the findings.

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