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NOVEL BIOMARKERS OF ATHEROGENIC DIET INDUCED DYSLIPIDEMIA AND METABOLIC SYNDROME SUPPRESSED BY *TERMINALIA ARJUNA*

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ABSTRACT: Atherosclerosis results from the slow deposition of fatty materials in media and large arteries leading to mortality worldwide. *Terminalia arjuna* is an herb of Combretaceae family which contains flavonoids, terpenoids, cardiac glycosides with high antioxidant properties. This study was conducted to determine the effect of hydroalcoholic extract fraction of *T. arjuna* on blood lipids and atherosclerosis in hyperlipidemia Wistar rats model induced by atherogenic diet. In the hyperlipidemia animal model, the rats received a hydroalcoholic extract of *T. arjuna* treatment exhibits a noticeable reduction in total cholesterol, total triglycerides and elevation in high-density lipoprotein cholesterol (HDL-C). A result reveals that *T. arjuna* considerably decreases TC, TG and increases antioxidant levels. Metabolic marker of homocysteine, pro-inflammatory (adipocytokines) markers such as C-reactive protein, leptin, and IL6, anti-inflammatory marker like adiponectin, fibrinolytic factors such as plasminogen activator (PA) and plasminogen activator inhibitor (PAI) that are an association with atherogenic diet consumption. Hence, *T. arjuna* extract can effectively prevent the progress of atherosclerosis. This is possible due to the effect of *T. arjuna* having triterpenoids, cardiac glycosides alkaloids, and flavonoids. The hydroalcoholic extract of *T. arjuna* was found to possess remarkable hypolipidemic activity.

INTRODUCTION: Atherogenic diets have distinct proatherogenic effects on gene expression and suggest a strategy to study the contribution of acute inflammatory response and fibrogenesis independently through dietary manipulation. A vast body of literature is available to demonstrate the various causative factors of early atherosclerosis and coronary heart disease. Endothelial dysfunction is the result of rapid development of atherosclerosis.

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Pro-inflammatory cytokines like TNF-, low level of endothelial nitric oxide and elevated level of C-reactive protein and Interleukins have shown a positive correlation with adverse cardiac events [1].

In addition to inflammatory markers, the formation of atherothrombotic plaques due to genetic and environmental factors produces a variety of metabolic, biochemical, & endocrine abnormalities among the prone individuals [2]. The tissue plasminogen activator (tPA) and urokinase are two important enzymes which convert plasminogen into plasmin. This enzyme is primarily produced by vascular endothelial cells. tPA is inhibited by PAI-I and hepatic clearance of tPA. The level of tPA is strongly associated with PAI-I activity. The tissue plasminogen activator inhibitor (tPAI) has been

recently emphasized for playing an important role in the removal of thrombi from the vascular system. It has been repeatedly observed that increased fibrinolytic activity includes adrenogenic agonist, vasopressin, bradykinin, and histamine. These factors significantly change the cardiac output, coronary blood flow, vascular elasticity and permeability of the essential constituents [3]. Further, it is also noticed that the Indian population has a high level of plasminogen activator inhibitor in comparison to western countries. Similarly, those individuals who have hypercholesterolemia show an elevated level of plasminogen activated inhibitor [4].

Both tPA and PAI-I have a strong correlation with triglycerides. Univariate regression analysis shows an elevated level of tPA and PAI-I. The limited demographic studies, conducted among Indian population have shown the high prevalence of coronary heart diseases (CHD) in the society where high levels of both enzymes are estimated [5]. It is also noticed that early atherosclerotic changes are more pronounced among chronic diabetes cases and cases of familial hypercholesterolemia and hyperglycemia, and hyperleptinemia. Therefore, it is considered essential to identify the instances of early atherosclerosis.

Hypercholesterolemia is the presence of a high level of cholesterol in the blood. It is not a disease but a metabolic de-arrangement that can be secondary to many diseases and can contribute too many forms of disease, most notably cardiovascular disease [6]. "Atherosclerosis" is the principle underlying cause of coronary heart disease, which is commonest cause of death in the industrialized world, and of stroke and peripheral vascular disease, which is also a significant cause of morbidity and mortality [7, 8].

Plant materials containing active compounds with chemically well-defined active substances, including chemically perfect isolated constituents of plants are not reflected to be herbal medicines [9]. Plant extracts isolated compounds are occasionally even more potent than known conventional drugs. It indicates that the researcher has stopped with just reporting the effect of plant chemical compounds and their outcomes are not interpreted into clinical research [10].

Taking these results advancing is compulsory to develop a new drug molecule. Therefore, further research into recognizing the active biomolecule, conducting preclinical and clinical studies is essential [7]. Recently, global attention has been focused on the utilization of herbal remedies for the prevention and management of various risk factors for CHD. Early identification of multiple risk factors may help launch preventive measures among the likely victims. The role of plant-based medicine has been recently emphasized by the World Health Organization. This organization has provided guidelines for evaluation of safety and efficacy profile of plant-based product [11].

Terminalia arjuna (Roxb.) is an Ayurvedic and Siddha plant with several medicinal values. It is commonly known as Arjuna, bark, which belongs to Combretaceae family comprising of nearly 200 species distributed around the world. Approximately 24 species of *Terminalia* have been reported from various parts of India; some selected species are *T. arjuna, T. bellirica, T. bialata, T. catappa, T. elliptical, T. porphyrocarpa, T. mantaly, etc.* In India, *T. Arjuna* is about 60 to 80 feet in height, buttressed trunk, and horizontally spreading crown and drooping branches distributed in Burma, Mauritius, India, and Sri Lanka.

It used for various complicated disease, treating wound healing, ulceration, lipid-lowering, astringent effect, urinary tract infection, regulate hormone cycles, airway clear lung disease, used for all kinds of bleeding, cardiac tonic and antioxidant [4, 11, 12, 13, 14, 15]. The active phytoconstituents of a fraction of *T. arjuna* include triterpenoids saponins, flavonoids, phenolic, and nutrients such as zinc, magnesium, copper, and calcium. Administration of *T. Arjuna* did not show any harmful adverse effect on hepatic, renal and hematological parameters (subasini *et al*., unpublished data). But the fraction of *T. arjuna* bark intense a high degree of anti-hyperlipidemic and anti-atherogenic and anti-oxidant activities. Moreover, the hydro-alcoholic fraction of *T. arjuna* shows that better effects of multi-targeted action of sitosterol as well as flavonoids action on the intestinal absorption of cholesterol and inhibiting HMG CoA enzyme respectively.

MATERIALS AND METHODS: *Terminalia arjuna* (TA) (Roxb.) bark is collected from Tirunelveli, Tamil Nadu, India This plant barks are authenticated in Rabinat Herbarium, St. Joseph College, Trichy, Tamil Nadu, India. The voucher specimens are deposited in the CARISM herbarium and maintained as TA- 0048. All the TA barks are shade dried for 15 days, and they are coarsely powdered using a pulverizer. Dried barks are subjected to hydroalcoholic extraction in the ratio of water: alcohol as 30:70, adopting a cold percolation method. The extracts are then, dried in vacuum and stored in a refrigerator. The preliminary phytochemical analysis revealed that hydroalcoholic fractions contained tannins, phenolics, sitosterol, anthraquinone glycosides, alkaloids, and flavonoids.

Atherogenic Diet: Atherogenic diet was rich in 30 % peanut oil and 5 % cholesterol. Atherogenesis is the formation of fatty materials plaque in the inner lining of epithelial cells of arteries, and it is associated with primary and secondary atherosclerosis. The atherogenic diet promotes atheromas; it was induced inflammation of epithelial cells permeability, which leads to leakage of LDL-C to the intima of arteries. Atheromas are the trademark of a cardiovascular disease called atherosclerosis.

Chemicals: Cholesterol, triglycerides, HDL cholesterol, were procured from SRL SISCO laboratories, Mumbai. Leptin was procured from Randox laboratories, and adiponectin chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA. Hs-CRP Apo-B Roche Diagnostic kits were purchased from SPINREACT and Himedia laboratories, Mumbai. All the other reagents used were of analytical grade. Standard drug - Atorvastatin's was purchased from the pharmacy shop.

Animals & Experimental Protocol: Pre-clinical study has been carried out to evaluate the efficacy profile of TA in reducing the triglycerides. Accordingly, 6 Albino rats of Wistar strain with an average body weight of 220.65 ± 18.75 g are taken for the present trial. The age of the rats varied from 6-8 weeks. The rats are housed in polypropylene cages at room temperature. All animals are kept in one room and no other species being housed with them. The room is well ventilated with fresh air and normal temperature. The animals are distributed randomly into different groups as per protocol.

Lipid profile and various pro-inflammatory markers like CRP, homocysteine, leptin, adiponectin, plasminogen activator, and plasminogen activator inhibitor that are in association with atherogenic diet consumption are measured. All animals had free access to water and standard pelleted laboratory animal diet.

This study was reviewed and approved by the Institutional Animal Ethical Committee (Reg. no. 817/08/ac/cpcsea). The study is divided into 4 groups. First group normal control (normal laboratory diet), the second group was atherogenic diet (rich in 30% peanut oil and 5% cholesterol), and the third group was treated with atherogenic diet + hydroalcoholic extract fraction of TA (25 mg/kg/ day). The fourth group was treated with an atherogenic diet + atorvastatin drug (0.4 mg/kg/day).

Blood is taken by the heart puncture just before the killing. The blood samples are centrifuged, and plasma is separated and frozen at – 80 ◦ C until needed for the assay.

Assay Methods: The total cholesterol, triglyceride, C-reactive protein assessed by immuno-turbidometric method (using RANDOX, UK kits - catalog no. CH 201) using cholesterol oxidase-PAP assay. Apolipo (B) and hs- CRP, assessed by immune assay method on a Hitachi modular analyzer. Serum adiponectin level, IL6 and leptin metabolism using ELISA assay, homocysteine level were measured using DPC kits on immunity-2000 autoanalyzer. Plasminogen activator inhibitor, tissue plasminogen antigen is measured by enzyme-linked immunosorbent assay (ELISA) [16].

Estimation of (TBARS): The levels of lipid peroxidation in tissues were estimated by the method of [17]. Assay of catalase (CAT, EC. 1.11.1.6). The activity of catalase was determined by the method of [18]. Assay of glutathione peroxidase (GPX) (GPX, EC 1.11.1.9) - Glutathione peroxidase was estimated by the method of [19]. Estimation of reduced glutathione (GSH) was estimated by the method of [20].

Calculations: Statistical analysis is performed using students 't' test by SPSS software 9.05. Results are expressed as mean ± SD. from six rats in each group. P values <0.05, 0.01 are considered as significant.

RESULTS:

Lipid Profile: The efficacy profile of hydro-alcoholic extract fraction of *T. arjuna* is evaluated in experimental animals. A comparative study is conducted with the standard drug is given along with atherogenic diet in one group of 6 rats, and 6 rats are treated with atherogenic diet along with *T. arjuna* for a period of 30 days. A group of 6 animals is served as normal control, and 6 animals are kept on an only atherogenic diet. This study has suggested that there is a drastic reduction in the various contents of lipid profile i.e. total cholesterol and triglycerides following conventional treatment statin [21]. The pattern of reduction in total cholesterol, and triglycerides content following *T. arjuna* treatment along with atherogenic diet treated animals is also significant, but the difference is not so marked, as it is in conventional treatment group **Table 1** and **2**.

But keeping the adverse effect of standard drug statin, we have to move the phytopharmaceuticals. Drugs, *T. Arjuna* is a better choice of drug for the management of hyperlipidemia responsible for type 4 atherosclerosis and endothelial dysfunction manifesting in ischemic heart disease [18]. So many authors result displayed that *T. arjuna is* having powerful hyperlipidemia and antioxidant properties due to rich constituents of secondary metabolites such as triterpenoids, tannins, flavonoids, and glycosides.

TABLE 1: ROLE OF HYDRO-ALCOHOLIC FRACTION OF TA ON TGL LEVEL AMONG EXPERIMENTAL ANIMALS

Groups	No. of Animals	Triglyceride (mg/dl)		
		Initial	After 15 days	After 30 days
Normal control	6	39.60 ± 7.98[a]	37.80 ± 8.60[a]	41.11 ± 5.26[a]
Atherogenic diet	6	44.58 ± 7.80[b]	589.66 ± 88.95[d]	648.65 ± 92.01[d]
Atherogenic diet + TA	6	40.82 ± 6.85[a]	180.54 ± 30.01[c]	210.25 ± 36.30[c]
Atherogenic diet + Statin	6	47.68 ± 6.80[c]	85.75 ± 14.82[b]	90.75 ± 16.85[b]

[a, b, c, d], values not sharing a common letter are significant differences between the groups ($p<0.05$).

TABLE 2: ROLE OF HYDRO-ALCOHOLIC EXTRACT OF TA ON TC LEVEL AMONG THE EXPERIMENTAL ANIMALS

Groups	No. of Animals	Total cholesterol level (mg/dl)		
		Initial	After 15 days	After 30 days
Normal control	6	64.95 ± 8.52[a]	70.51 ± 6.38[a]	78.62 ± 7.66[a]
Atherogenic diet	6	70.10 ± 6.90[b]	120.35 ± 19.88[d]	180.42 ± 20.90[d]
Atherogenic diet + TA	6	68.46 ± 5.01[ab]	110.85 ± 20.60[c]	130.65 ± 30.85[c]
Atherogenic diet + Statin	6	78.77 ± 7.82[c]	90.32 ± 11.11[b]	100.40 ± 22.38[b]

[a, b, c, d], values not sharing a common letter are significant differences between the groups ($p<0.05$).

Apolipo B & Homocysteine: In the present, experimental series, the apolipo B content was significantly increased following the atherogenic diet group of animals. Following 30 days of *T. arjuna* and statin treatment which increase in the level of Apolipo B was not so significant changes when given along with atherogenic diet. Thus, the anti-atherogenic property of *T. arjuna* is proven in **Fig. 1A**. The non-lipid factor of homocysteine level, when measured in experimental rats, has also shown elevated level in the group-II whereas atherogenic diet was given and the homocysteine level was increased significantly in group-III and IV where the animals were fed atherogenic diet and treated with the drug *T. arjuna* and statin. The more beneficial effect was observed in the statin treatment group of animals **Fig. 1B**. A similar report was also published in the literature [22].

Antioxidant Effect: Table 3 shows the antioxidant activity of the drug in heart homogenate after 30 days. The antioxidants such as catalase, GSH, and GST level were significantly decreased ($p<0.05$) in the heart of disease induced rats when compared to normal group. Then these levels are significantly increased ($p>0.05$) in the drug (TA) treated groups. But the level of TBAR showed the reverse effect of the above parameters. The level is significantly decreased ($p<0.05$) after the administration of the drug. The fourth group with statin showed significant results when compared with the 2nd group of atherogenic diet.

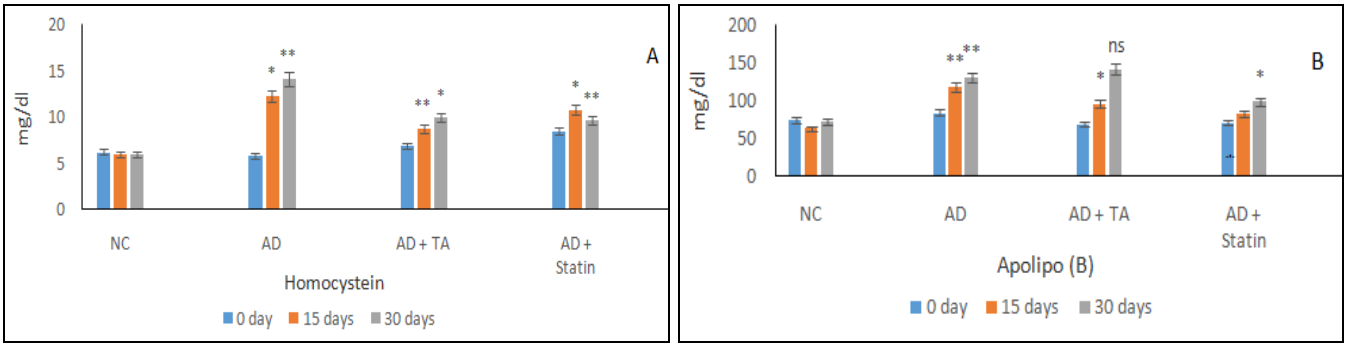

FIG. 1: EFFECT OF TA ON HOMOCYSTEINE LEVEL (A) & APOLIPO B (B) AMONG ATHEROGENIC DIET TREATED RATS. Student t test – P<0.05*, P<0.01**

TABLE 3: EFFECT OF TA FRACTION IN HEART ANTIOXIDANTS OF ATHEROGENIC DIET INDUCED HYPERLIPIDEMIC RATS

	TBARS	Catalase	GSH	GST
Normal control	0.34 ± 0.15^{b}	33.39 ± 3.35^{c}	2.3 ± 0.02^{d}	1.2 ± 0.01^{c}
Atherogenic diet	1.16 ± 0.01^{d}	1.34 ± 0.01^{a}	1.5 ± 0.01^{a}	0.6 ± 0.03^{a}
Atherogenic diet + TA	0.49 ± 0.03^{c}	12.56 ± 9.65^{b}	1.8 ± 0.02^{b}	0.9 ± 0.01^{b}
Atherogenic diet + Statin	0.25 ± 0.061^{a}	42.90 ± 4.05^{d}	2.1 ± 0.03^{c}	1.1 ± 0.02^{c}

[a, b, c, d], values not sharing a common letter are significant different between the groups (p<0.05). TBARS-(nM of malondialdehyde/mg of protein, Catalase- nM of H_2O_2 used/min/mg of protein) (µM of GSH consumed/min/mg of protein, GHS- (µg of GSH/mg of protein), GST-(nM of H_2O_2 used/min/mg of protein) (nM CDNB-GSH conjugate formed/min/mg of protein).

Specific enzymes such as superoxide dismutase, catalase, and glutathione peroxidase can protect the organism against the reactive oxygen species effects [23].

Pro-inflammatory Markers: The result was obtained out of the study indicates that the inflammatory markers like C-reactive protein and interleukin-6 significantly increased after 15th and 30th days following atherogenic diet consumption among the rats. But the level of CRP and IL6 was quite less in the group III where the atherogenic diet was given along with *T. arjuna* and standard drug [20, 28]. The trend of increase in the level of CRP was significantly less in the standard group of drug when compared to *T. arjuna* group of animals **Fig. 2A** and **Fig. 2B**.

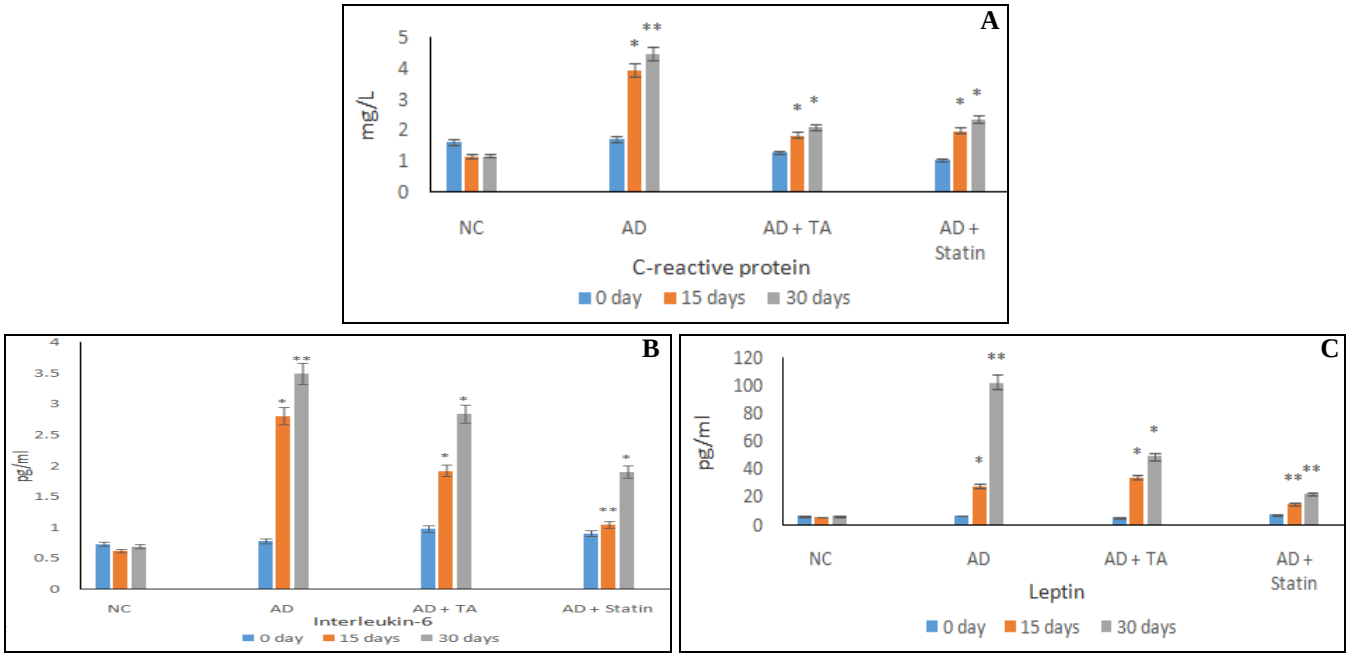

FIG. 2: EFFECT OF TA ON PRO- INFLAMMATORY MARKERS (A, B & C) AMONG ATHEROGENIC DIET INDUCED RATS. Student t test – P<0.05*, P<0.01**

Leptin: Fig. 2C summarizes the blood leptin level in the atherogenic diet was given for 30 days continuously to the experimental rats. A significant reduction of leptin level was measured in group III and group IV. Whereas, the II group of animals have a high level of leptin when compared with normal group of animals. These results were suggested that *T. arjuna* have a potential anti-obesity and antiatherogenic effect [24, 25].

Anti-inflammatory Markers: Adiponectin, when estimated in this series of experimental study, has exerted an inverse relationship with atherogenic diet consuming rats. A decrease in the level of adiponectin was noticed following an atherogenic diet that has increased under the influence of *T. arjuna* and a standard group of animals. The result shows that decrease in adiponectin is significantly less where the animals were treated with drugs along with atherogenic diet. The more beneficial role was noticed in the statin group of animals than the *T. arjuna* treated animals in **Table 4**. *T. arjuna* having an antioxidant property which stimulates the protein hormone, it may lead to increase the level of adiponectin which might reduce the inflammation [26].

TABLE 4: ANTI-INFLAMMATORY MARKERS - ADIPONECTIN LEVEL ON TA TREATMENT IN ATHEROGENIC DIET INDUCED RATS

Groups	No. of Animals	Adiponectin (µg/L)		
		Initial	After 15 days	After 30 days
Normal control	6	48.95 ± 10.80^{c}	53.32 ± 9.01^{c}	50.35 ± 8.33^{c}
Atherogenic diet	6	42.53 ± 12.85^{b}	30.75 ± 8.01^{a}	24.50 ± 5.48^{a}
Atherogenic diet + TA	6	48.75 ± 10.85^{c}	40.75 ± 9.95^{b}	48.20 ± 8.35^{c}
Atherogenic diet + statin	6	39.85 ± 7.82^{a}	38.85 ± 8.01^{b}	43.85 ± 10.85^{b}

[a, b, c, d], values not sharing a common letter are significantly differences between the groups ($p<0.05$).

Fibrinolytic Factors: The fibrinolytic factors like tissue plasminogen activator and plasminogen activator inhibitor have shown elevated level among hypertriglyceridemic animals. *T. arjuna* slightly reduces the elevated level of TPA and PAI at 30 days' treatment. *T. arjuna* treatment group animals do not show any significant value for PAI at 30 days. Both *T. arjuna* and standard drug statin treatment does not reduce the rise in the level of TPA at 15 days which was displayed in **Fig. 3A** and **3B**.

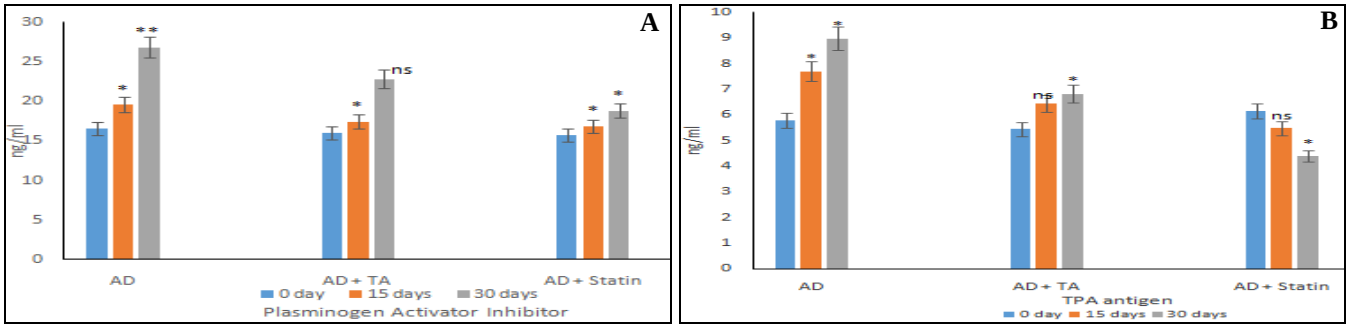

FIG. 3: EFFECT OF TA ON PAI (A) & TPA (ANTIGEN) LEVEL (B) AMONG EXPERIMENTAL ANIMALS. Student t test – $P<0.05$*, $P<0.01$**

DISCUSSION: The experimental study demonstrated that diet-induced hyper-triglyceridemic animals to have an elevated level of homocysteine and pro-inflammatory markers like C-reactive protein, leptin, and interleukins (IL6). The release of leptin from adipose tissue is a negative feedback mechanism to quash the appetite and reduction in weight gain. The cardiac markers including total cholesterol, triglycerides, and C-reactive protein levels are maintained normal. We have also estimated the level of TBARS the marker of antioxidants in atherogenic diet administered animals. Since oxidative stress is the major factor responsible for the development of age-related diseases and other cardiovascular diseases. The antioxidant activity was increased by increasing the activity of enzymatic antioxidants like GST and catalase, non-enzymatic antioxidants like GSH along with the decrement of TBARS.

Atherogenic dyslipidemia is a part of the complex cluster of abnormalities called the metabolic syndrome which has a direct correlation with coronary artery disease (CAD) events. In recent years, Indian population has increased incidence of atherosclerosis disease (AD) and CAD as compared to western population, which may be due to a lifestyle changes such as physical inactivity, unhealthy diet, and a higher genetic predisposition. Despite large reduction in LDL-C levels, significant residual cardiovascular risk due to low HDL-C, high TG, and non-HDL-C levels exist. Their management with the therapeutic lifestyle changes with statins or statins combination with niacin and fibrates has considerably reduced the incidences of cardiovascular disease (CVD) events. Apo B/ApoA-1 ratio has been considered as an accurate predictor of (CVD) risk, however, several studies have reported LDL-C/HDL-C ratio to be more accurate.

The hydro-alcoholic fraction of *T. arjuna* fraction has shown a significant effect on triglycerides, and inflammatory markers particularly involved in endothelial dysfunction resulting in atherosclerosis, hypertension and ultimately leading to a myocardial infarction and cerebrovascular accidents. Currently, the following atherosclerotic factors have been recognized by the genetic inheritance particularly lipoprotein (a), and leptin has been recently recognized as one of the important hormones responsible for insulin resistance, endothelial dysfunction and, the extra formation and deposition of adipose tissues. Hyperleptinemia has shown significant metabolic effect, particularly on the lipid metabolism. Elevated level of leptin is responsible for high production of glucocorticoids resulting in an elevated level of plasma cortisol. The endocrine effect starts from hypothalamus involving complete hypothalamo-hypophysial neuro-endocrine axis. Leptin plays an important role in the regulation of food intake, energy expenditure, lipid metabolism, and immune function. It is associated with metabolic syndrome, dyslipidemia, and inflammatory markers.

Interleukin is a novel adipokine pro-inflammatory marker, IL6 concentration raises with a weight gain of atherogenic animals. IL6 concentration reduced with weight loss of atherogenic animals, which disturb appetite and energy intake through action in the hypothalamus. Adiponectin is the important adipocyte complement-related protein regulating the glucose and adipose tissue homeostasis. It is also responsible for anti-atherogenic effects and anti-inflammatory effects, neutralizing the adverse effect of hyperleptinemia and hyperglycemia. In a series of experimental studies, it has been observed that atherogenic heart animals have a low level of adiponectin. Similarly, adiponectin is also responsible for enhancing the pro-inflammatory cytokines as leptin level increased in experimental models. Adiponectin is a cardiovascular protector; it also inhibits endothelial cell, ventricular walls, smooth muscles, production of adhesion molecules responsible for endothelial damage.

The level of homocysteine, a sulfhydryl-containing amino acid has shown to be predictive of future coronary heart disease (CHD). There is also evidence that the elevated range of homocysteine has a significant association with atherosclerosis and the formation of atherothrombotic plaque responsible for ischemic heart disease. It has been observed in several cases and also among the patient suffered from a vascular disease that genetic factor influences plasma homocysteine concentration. It is also reported that plasma homocysteine rises with age in both men and women and its concentration are higher in men than women. This may be due to the difference in muscle mass and renal function. Sex hormones may also influence homocysteine concentration in plasma [29].

The elevated plasma homocysteine is a known factor for atherosclerotic vascular disease. Homocysteine level increased with decreasing concentration of vitamin B_6, vitamin $B_{12,}$ and folate. Further, it increases due to impaired metabolism by the kidneys and liver which enhances the risk of myocardial infarction and stroke [30]. Certain drugs like a combination of colestipol and niacin, methotrexate, phenytoin carbomazepine, and nitrous oxide may increase homocysteine concentration in plasma. Further, it is pointed out that patients with inherited defects of methionine metabolism can develop severe hyperhomocysteinemia and can have premature atherothrombosis. Though, mild to moderate elevations of homocysteine are common among the

general population, due to insufficient dietary intake of folic acid, vitamin B_6 and B_{12} level, if the condition is not treated, it may cause endothelial dysfunction and formation of atherothrombotic plaque.

Recently, hyperhomocysteinemia has been recognized as an independent risk factor for cardiovascular disorders [31]. It is further, noticed that hyperhomocysteinemia is threefold more common in cases suffering from type-II diabetes mellitus [32]. More recently, several studies have been demonstrated in such a way to attest that elevated level of homocysteine is responsible for endothelial dysfunction, the formation of atherothrombotic plaque and also responsible for neurodegeneration. In the present thesis, the researchers had included the role of hyperhomocysteinemia in the causation of atherosclerosis and the remedial measure for its prevention. Early estrogen deficiency in females is responsible for abnormal lipid profile as well as an increased atherosclerotic process. Hormone replacement therapy may significantly prevent the abnormal elevation of lipids as well as the reduction in the vascular inflammatory process responsible for the rapid progression of atherosclerotic changes [33]. But prolong administration of HRT may precipitate the breast and endometrial cancer.

Oxidative stress is responsible for endothelial dysfunction in different age and sex group. In type-II diabetes mellitus, oxidative stress occurs through the formation of reactive oxygen species and lowers the anti-oxidant concentration [34]. The mechanism of oxidative injury responsible for endothelial dysfunctions are complex and varied but in different experimental and clinical studies [35], it is observed that oxidative stress may accelerate the information process and responsible for rapid atherosclerotic changes. A vast body of evidence is available to indicate the role of anti-oxidant in the prevention and management of endothelial dysfunction in type-II diabetes mellitus [36].

CONCLUSION: In the present experimental studies, the researcher has observed that the herb *T. arjuna* has adiponectin enhancing property among atherogenic diet-induced rats. Atherogenic diet is responsible for hyper-triglyceridemia leading to endothelial dysfunction due to the enhanced vascular inflammatory process. High level of C-reactive protein, interleukin-6 has a significant effect in reducing the adiponectin and thus, enhances the atherosclerotic process that may be manifested in a future cardiac event. *T. arjuna* has shown significant elevation of adiponectin among the animals showing evidence of hypertriglyceridemia. *T. arjuna* has shown the potential effect on total cholesterol by acting on cholesterol receptors as well as reducing the oxidized high LDL cholesterol. Likewise, the same *T. arjuna* fraction also displays the antioxidant activity by increasing the activity of enzymatic antioxidants like GST and catalase, non-enzymatic antioxidants like GSH along with the decrement of TBARS. It increases adiponectin level and decreases pro-inflammatory adipokines, proving its anti-atherogenic property. The reduction in inflammation and cell damage may be due to antioxidant and anti-lipid peroxidative effect

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