



POTENCY OF ETHANOLIC EXTRACT OF *TECOMA STANS* ON cIMT AND MDA ON RATS EXPOSED TO CIGARETTE SMOKE

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Abstract: The goal of this research was to determine the atheroprotective effect of *Tecoma stans* ethanolic flower extract (EETS) against intima-media carotid artery thickness (cIMT) and malondialdehyde level (MDA) on rats exposed to cigarette smoke. Thirty adult Wistar rats in group K2, K3, and K4 (100 mg / kg b.w / day; 200 mg / kg b.w / day; and 400 mg / kg b.w / day) were divided into five groups and exposed to cigarette smoke, 3 pc cigarettes / day, respectively. In both participants the treatment period was 28 days. Blood was taken out of the heart to assess the amount of MDA. To assess cIMT, histological slide was obtained from carotid artery intima-media. The administration of EETS could substantially prevent the development of atherosclerosis due to oxidative stress by decreasing the MDA level ($p < 0.05$) and decreasing the degree of changes in cIMT ($p < 0.05$). The present study found that EETS could prevent the development of atherosclerosis due to smoke exposure by reducing the level of MDA, that is, the marker of oxidative stress associated with reduced changes in cIMT. Further researches on additional EETS bioactivity as an antioxidant are therefore required for the invention of potent atheroprotective drug.

Index Terms - *Tecoma stans*, Malondialdehyde, cIMT, Atherosclerosis, Smoke exposure.

I. INTRODUCTION

Cardiovascular disease (CVD) is the main cause of death in developing and developed countries alike. Coronary artery disease occurs in just as much 42 percent of males and 25 percent of females, with an even higher percentage of other complicated individuals. It is estimated that as many as 23.3 million people will die from CVD by 2030 (Andhuvan G et al. 2016), (Thomas S and Rich MW 2007). Oxidative stress, which is an imbalance between oxidants and antioxidants inside the body (Saxena M et al. 2012) is one of the main causes of this disease. There is evidence that oxidative stress plays an important role in the pathogenesis and development of CVD, including atherosclerosis (Saxena M et al. 2012), (Grassi D et al. 2010).

Atherosclerosis is a systemic disease which is responsible for most CVDs and stroke incidences (Coll B and Feinstein SB 2008). Atherosclerosis is a complex phenomenon that is the main cause of coronary artery disease, which can be illustrated as an excess of fibro-fatty substance, or as an inflammatory response to arterial wall damage (Libby P et al. 2011), (Mahdavi-Roshan M et al. 2013). Cigarette smoke exposure is one of the major risk factors for the early development of atherosclerosis and other complications of CVDs. Nicotine, tar, and carbon monoxide are some principal components of cigarette smoke. Such drugs can have a detrimental effect on the body, on both the respiratory system and the cardiovascular system (MiYang J et al. 2014).

Smoke from cigarettes also contains some free radicals. These free radicals will initiate the creation of reactive oxygen species (ROS), which are reactive molecules constantly produced inside the body by the enzymatic reactions (Valavanidis A et al. 2009), (Dellinger B et al. 2011). If the ROS level rises beyond that of the antioxidant defense mechanism in the body, oxidative stress occurs (Valavanidis A et al. 2009), (Dellinger B et al. 2011). Oxidative stress produces substances which can induce cytotoxicity in the blood vessel's endothelial cells and smooth muscles, including many variants of aldehyde, one of which is malondialdehyde (MDA) (Moore KP et al. 1995), (Ballinger SW et al. 2000).

Oxidative stress is reported to play an important role in the development and initiation of atherosclerosis by stimulating the production of inflammatory factors and cytokines (Fernández-Robredo P et al. 2008). Atherosclerosis is characterized by weakening of the vessel walls, inflammatory response, foam cell plaque formation and blood clot formation. The oxidative stress cycle induces endothelial dysfunction, while endothelial dysfunction increases the state of constant oxidative stress, thus causing atherosclerosis to develop (Grassi D et al. 2010)

This modification of the endothelial function plays a significant role in atherosclerosis pathophysiology (Barakoti M 2018). A published research on the topic has succeeded in proving the presence of foam cells in the blood vessels, from the tunica intima to the tunica media in the aorta of rats exposed to cigarette smoke for 20–30 days (Lapatta N et al. 2013). The early phase of atherosclerosis is the alteration response of medial cells that can be measured by measurement of carotid artery intima-media thickness (cIMT), and later stages are the generation of carotid plaques (Barakoti M 2018). Measurement of the common carotid artery intima-media

thickness (IMT) is an early morphological marker for the incidence of atherosclerosis (Coll B and Feinstein SB 2008). IMT is an indicator of risk factors for atherosclerosis and the treatment used to reduce the influence of those risk factors (Moore KP et al. 1995), (Barakoti M 2018), (Halenka M et al. 2004).

Consumption of foods dependent on vegetables is also associated with low risk of CVD and hypertension. Several research studies have focused on secondary metabolic substances from plants, such as flavonoid, in recent decades as a substance that can help prevent CVDs (McCullough ML et al. 2012), (Suhartati S and Khaerunnisa S 2016). *Tecoma stans*, also known as yellow trumpet bush, belongs to the bignoniaceae family. It's an ornamental weed. It is a two to four meter thick erect, branched, sparsely hairy or almost smooth shrub. The leaves are opposite, odd-pinnate, with 5 to 7 leaflets, up to 20 centimeters in length. The leaflets are lanceolate to oblong, 6 to 13 centimeters long, pointing at both ends and dented at the margins. Trumpet-shaped flowers are mildly scented yellow and borne in small, dense, terminal clusters. The limes are white. 5 to 7 mm long and 5 toothed. Blooming can start as early as April and continue to fall in. Following the flowers are 6 inches long tan pods packed with tiny, papery winged seeds (Parrotta JA 2001)

Tecoma stans leaves contain the alkaloids tecomin and when given intravenously, tecostamine is a potent hypoglycaemic agent. Anthranilic acid is responsible for the action against diabetics. Diuretic and vermifuge roots are powerful (KNV Rao et al. 2010). *Tecoma* is not a poisonous plant as it is used in Latin America as a diabetes cure and, respectively, in Mexico (Khare CP 2007) to feed cattle and goats. *Tecoma stans* preliminary phytochemical screening of ethanolic floral extract showed the presence of flavanoids, phenol, alkaloids, tannins, steroids, triterpenes, anthraquinones, and saponins etc.

This work was conducted to examine the atheroprotective effects of EETS as a potential early preventive measure against CVD due to cigarette smoke exposure, assessed by observed histopathological changes of the cIMT and oxidative stress parameters calculated through the presence of MDA methods.

II. MATERIALS AND METHOD

Collection and extraction of plant

The *Tecoma stans* flowers were collected from Rasipuram [Namakkal District] Tamil Nadu in the month of May 2019. A plant herbarium specimen had been deposited at the Pharmacognosy Section. Dr. G.V.S. Murthy, Joint Director of the Indian Botanical Survey, Southern Circle, TNAU Campus, Coimbatore, described the plant from the details available in the literature. During 10–12 days the flower petals were dried in the shade. After full drying in a mechanical grinder, the flower petals were pulverized to a coarse 40-mesh powder. The powdered material was subjected to extraction of Soxhlet with ethanol at 50–55 °C for 18 h. The extract was subsequently concentrated under vacuum, and dried air (Mukherjee, K.P 2004), (Kokate C. K 2004), (Evans C.W 2002).

Experimental design (Kandimalla R et al. 2015)

The Wistar rats were divided into five classes, acclimatized to the laboratory conditions and maintained at room temperature under 12 h light and dark cycles. The animals were given a standard diet and water ad libitum: K0: negative control group, K1: positive control group (smoke exposure group), K2: treatment group 1 (EETS 100 mg / BW smoke exposure and ethanol extract, K3: treatment group 2 (EETS 200 mg / kgBW smoke and ethanol extract, and K4: treatment group 3 (EETS 400 mg / kgBW smoke and ethanol extract.

The carotid artery IMT (cIMT) measurement:

The isolated carotid artery tissue is set at 10 percent with formalin. It is then converted into a histological slide and treated with coloring by Hematoxylin Eosin (H&E). Observation is done with a microscope of Olympus, with 0.100. The IMT is analyzed using the cellSens program to measure the tunica intima and media from the intimate endothelial boundary to the outermost tunica media.

The measurement of malondialdehyde levels (MDA)

The MDA amount is obtained from the Wistar rat serum and is determined using the reactive thiobarbituric acid method and observed with a spectrophotometer measured in nmol / ml at a wavelength of 529 nm (Mohamed O et al. 2016). MDA amount was obtained with Cat No.0801586 from Zeptomatrix, Buffalo, New York, United States.

Cigarette smoke exposure Cigarette smoke exposure in experimental animals was provided by 3 pc cigarettes a day in groups K1, K2, K3, and K4 for 28 days, at 6 p.m. The exposure device consisted of a two-hole glass box; some holes for cigarette smoke injection, and other holes for cigarette smoke removal. To suck the cigarette held on the iron pipe, a 50 cc syringe is taken out, so the cigarette smoke has reached the syringe. The 50 cc syringe pumping process is performed repeatedly until the cigarette has burnt out. This analysis utilized the locally available cigarette brand, manufactured in Surabaya, East Java, Indonesia.

Statistical Analysis

The results are expressed as mean values \pm S.E.M. [standard error of mean] for pairs of rats. Statistical comparison was carried out by analysis of variance [ANOVA]. The difference between the means of treated groups and the non-treated control group was evaluated by the Bonferroni Multiple Comparisons. The results were considered statistically significant when $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 summarizes the average outcome of cIMT measurement in control and experimental animals as substantial increase ($p < 0.05$) in cIMT was observed in group K1 (rats exposed to cigarette smoke) compared with group K0 (control group rats). EETS administered rats (Group K2, K3, and K4) reported a substantial decrease in their cIMT ($p < 0.05$) relative to group K1 rats, which indicates the substance's atheroprotective activity against exposure to cigarette smoke.

Table 1: Mean, standard deviation, and ANOVA test of carotid artery intima-media thickness of rats control and treatment groups.

Parameter	Groups					SI	ANOVA
	K0	K1	K2	K3	K4		
cIMT	31.45±4.21 ^a	44.86±6.83 ^{b,d}	37.32±4.22 ^{a,c}	39.07±1.24 ^{a,d}	40.24±9.23 ^{c,d}	µm	0.022*

*Significantly with $p < 0.05$, #Mean±SD, a,b,c,d Different superscript means significant between groups. cIMT: Carotid artery intima-media thickness, SD: Standard deviation, SI: System International of unit.

The amount of MDA was observed from the serum using TBARC procedure. Table 2 indicates the degree of MDA in the different groupings. In Group K1 (rats exposed to cigarette smoke), a substantial increase ($p < 0.05$) in MDA levels was observed relative to rats belonging to Group K0. EETS-administered rats (i.e., K2, K3, and K4) reported a reduction in their MDA levels compared to Group K1. It indicates the EETS antioxidant activity against exposure to tobacco smoke.

Table 2: Mean, median, minimum, maximum, and Kruskal–Wallis test of malondialdehyde of rats control and treatment groups

Parameter	Category	Groups					SI	Kruskal–Wallis
		K0	K1	K2	K3	K4		
MDA	Mean	1.71 ^a	5.74 ^b	2.34 ^c	3.29 ^{b,c}	3.43 ^c	nmol/ml	0.002*
	Median	1.68	6.74	2.19	2.84	2.11		
	Minimum	1.40	2.66	2.00	1.84	1.86		
	Maximum	2.09	8.00	3.00	5.19	6.32		

*Significantly with $p < 0.05$, #Mean±SD, a,b,c,d Different superscript means significant between groups. cIMT: Carotid artery intima-media thickness, SD: Standard deviation, SI: System International of unit

The carotid artery sample histochemistry was used for determining cIMT. An early morphological marker for the onset of atherosclerosis is the common carotid artery IMT. Research shows that cigarette smoke exposure greatly affects the cIMT when compared with the normal group. EETS administration may avoid a smoke-derived cIMT modification, which is not substantially different compared to the positive control group compared to the usual group

The present study showed that EETS administration on cigarette smoke-exposed rats could prevent the development of atherosclerosis by reducing the level of MDA and degree of cIMT changes in the samples. Analysis of phytochemistry screening found that the ethanol EETS contains flavanoids, phenol, alkaloids, tannins, steroids, triterpenes, anthraquinones and saponins suggested to play a significant role in the present study as atheroprotective.

Pathogenesis of atherosclerosis due to smoke exposure (based on an oxidative stress study)

Previous study suggested that active and passive smoking of cigarettes is associated with endothelial cell dysfunction (Mazzone P et al. 2010). Cigarette smoke contains some free radicals that can initiate ROS generation, which are reactive molecules constantly produced by the body's enzymatic reactions (Valavanidis A et al. 2009) (Dellinger B et al. 2011). ROS compounds are formed by several oxidase enzymes, including nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS), cyclooxygenase (COX), glucose oxidation, lipoxygenase, and withochondrial electron transport (Hertzog DI and Tica OS 2012). The disease pathology mechanism involved a variety of ROS and reactive nitrogen species (RNS), including radical superoxides (O_2^-), radical hydroxyl ($OH\cdot$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), radical hypochlorite (CLO), heme ferryl species protein, alkoxy lipid (RO) and radical peroxy (ROO), radical peroxy nitrite ($ONOO\cdot$), nitrite oxide (NO), and radical nitrite (Grassi D et al. 2010), (Patel JM. 2010), (Halliwell B 2006), (Stefek M 2011).

The imbalance within the human body between oxidants and antioxidants has the ability to cause harm through a mechanism called oxidative stress. This is a metabolic disorder, endogenous, biological, or organism-wide, characterized by an oxidative overload. The means by which ROS disturbs cellular function cannot yet be completely explained; however, the occurrence of lipid peroxidation, which induces cell death, is one of the most important mechanisms (Patel JM. 2010), (Sandhar H et al. 2011). One of the lipid membrane degradation products is MDA which is provided by the lipid peroxidation cycle. This marker has frequently been identified in patients with atherosclerosis, certain types of cancers, neurodegenerative diseases and lung diseases, especially those caused by inflammation (Sandhar H et al. 2011).

Some current evidence suggests that oxidative stress plays a major role in the pathogenesis and growth of CVDs, including atherosclerosis (Grassi D et al. 2010). Atherosclerosis is an inflammation of the blood vessel due to the accumulation of atheromatous plaques within the artery walls which causes the endothelial cells of the vessel wall to dysfunction (Suhartati S and Khaerunnisa S 2016). Endothelial dysfunction causes oxidative stress, and endothelial dysfunction induces chronic oxidative stress. This forms a vicious cycle which ultimately leads to atherosclerosis. Oxidative stress is associated with a lack of tetrahydrobiopterin and a decrease in eNOS activity which can lead to endothelial dysfunction in atherosclerotic patients by causing an increase in the cycle of oxidative stress within the body (Grassi D et al. 2010).

The decrease in endothelial NO bioavailability can cause the following conditions in patients with a high cardiovascular risk level: (a) reduced eNOS expression; (b) disruption of cellular signals causing an eNOS substratum or cofactor deficiency and reduction of eNOs activation; (c) decrease in the endothelial cell's ability to synthesize and/or release NO; and (d) decrease of NO synthetic activation. All of these abnormalities may result in endothelial dysfunction, which is generally seen as an initial step in atherosclerosis pathogenesis (Grassi D et al. 2010).

The early phase of atherosclerosis, which can be measured by measuring carotid IMT (cIMT) and later stages, is mostly carotid plaque production. -- cIMT is usually seen because of its flow dynamics in a specific carotid artery, while plaques are particularly seen in

the internal carotid artery or carotid bulbs. As observed in current studies, elevation of cIMT is the most frequently associated vascular condition due to exposure to smoke (Bauer M et al. 2012).

Flavonoid; its function as an atheroprotective agent:

The atherosclerosis cycle has long been seen as a systemic chronic inflammatory disorder, causing morbidity and mortality primarily through its consequent cardiovascular diseases (McCullough ML et al. 2012). A high intake of fruit and vegetable flavonoids has a proven association with a lower risk for cardiovascular diseases. The mechanism for understanding the associated process is still uncertain, but current evidence clearly indicates that flavonoid can help to minimize risk factors for cardiovascular disease (Suhartati S and Khaerunnisa S 2016).

The flavonoid, which is included within the category of phenolic compounds, is frequently present in plant tissues and can serve as an antioxidant. The antioxidant function of flavonoid stems from its ability to contribute atoms of hydrogen, or its ability to chelate metal. Different work has shown that flavonoid (Bauer M et al. 2012.) has anti-allergic, anti-inflammatory, antimicrobial, anticancer, anti-diarrhea, and antiviral effects. Several studies have shown that a high flavonoid intake is positively related to a substantial reduction in risk of coronary heart disease.

The endothelial vascular cells play an important role in protecting the health of the heart by releasing nitric oxide, a drug that relaxes the arteries (causes vasodilatation). Endothelial nitric oxide development can inhibit platelet adhesion and aggregation, which is one of the preliminary factors in blood clot formation. Various clinical studies have examined the role of high flavonoid intake in reducing a multitude of platelet aggregation sizes; various findings have been reported in these trials. Food-based flavonoids have been documented to be potentially involved in the prevention of cardiovascular diseases, primarily through reducing oxidative stress and enhancing NO bioavailability. Flavonoid is intended to modulate genes linked to metabolism, stress-defense, the metabolism of proteins, detoxification, and protein transporters (Suhartati S and Khaerunnisa S 2016).

Most flavonoids act as effective free-radical scavengers. However, this effect may not always be helpful, since after completing their scavenging functions, flavonoids transform into radical flavonoids. Nevertheless, some high-stability radical flavonoids do not react easily, and will act as an antioxidant instead. The position and total number of hydroxyl chains in the flavonoid chemical structure, their shape, substitution and other factors contribute to their ability to chelate metallic ion, which in effect allows them to scavenge and inhibit free radicals (Hertzog DI and Tica OS 2012).

The hydroxyl chain pattern in the B-ring appreciably muffles (scavenges) and chelates metallic ion from ROS and RNS due to their role as hydrogen and electron donor to hydroxyl, peroxy, and radical peroxy chains, which produces comparatively stable radical flavonoids or radical aroxyl (FI-O.) (Patel JM 2010). Apart from this, flavonoid has an antioxidant influence that can increase a cell's length of life, along with induce apoptosis and prevent cell proliferation, which is why it can also act as an anticarcinogenic factor. It is reported that flavonoids (epigallocatechin-gallate and resveratrol) can restrain transcription factors like NFκB and AP-1 through an interaction between upstream signaling pathways (IKK phosphorylation, MAPK phosphorylation, and P13K/Akt phosphorylation) and/or by tumbling proinflammatory mediators (tumor necrosis factor-α, interleukin, and prostaglandin E2) and the action of pro-inflammatory enzymes (COX 2, iNOS) (Bauer M et al. 2012).

Flavonoid modulation of cell-signal pathways can help prevent cardiovascular disease by the following means: (a) reduced inflammation, (b) reduced expression of vascular-cell adhesion molecules, (c) increased activity of endothelial nitric oxide synthase (eNOS) and (d) reduced platelet aggregation; To keep the vasodilatation, nitric oxide is necessary. Nitric oxide disorders are also associated with an increased risk of CVDs; (d) decreased platelet aggregation: the aggregation of thrombocytes is one of the first steps in blood clot formation. Such clots can block coronary or brain arteries and cause either a myocardial infarction or a stroke. Aggregating platelets is an effective technique in the main and secondary prevention of CVDs (Suhartati S and Khaerunnisa S 2016).

Our study suggested, based on the evidence from the current results, that the health-promoting effects of EETS on these animal models of rats exposed to cigarette smoke could be due to the atheroprotective effects. As a preventive against atherosclerosis, we suggest that EETS can direct free radical interception before any significant oxidation that was suggested by decreasing MDA levels can occur. As an antioxidant, EETS will inhibit or delay the oxidative processes leading to a decrease in MDA, i.e., lipid peroxidation end product. Reduces the incidence and avoids atherosclerosis by inhibiting changes in carotid artery IMT caused by exposure to cigarette smoke.

IV. CONCLUSION

The results of this study showed that exposure to cigarette smoke could increase the level of MDA, i.e., marker of oxidative stress and degree of changes in the experimental animal's carotid artery IMT (cIMT). EETS-administered ethanol extract showed a strong antioxidant capacity for lowering the level of MDA and thereby reducing the production of atherosclerosis, which was shown by decreasing the degree of cigarette smoke-induced changes. Additional studies on other EETS bioactivity are however warranted.

V. REFERENCES

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